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Environmental Physiology of  
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## Introduction

Cherimoya is said to be the most delicious of the *Annona* fruit (Purseglove, 1968; Morton, 1987). The cherimoya is relatively expensive owing to its scarcity value. The optimum area for this fruit cultivation is so limited that the production is not sufficient to satisfy the increasing worldwide market demand. Moreover, the long transport of cherimoya is difficult because of the short shelf life even under low temperature condition. Cherimoya, sugar apple, and soursop are of major importance in *Annona* trees in commercial and subsistence agriculture. In these fruit trees, cherimoya cultivation is considered to be the most expectable owing to its delicious taste. Despite this fact, very little information is available on environmental physiology of this tree, although some studies have been reported on atemoya, the hybrid of cherimoya and sugar apple.

### ***Origin and distribution***

The cherimoya is believed to be indigenous to the inter-Andean valleys of Ecuador, Colombia and Bolivia (Morton, 1987). In Colombia and Ecuador, it grows naturally at elevations between 1400 - 2000 m where annual mean temperature ranges between 17°C and 20°C. The monthly mean temperatures in Peru lie between 18°C and 25°C in summer and 18°C and 5°C in winter. Annual rainfall in Pichincha district, a large cherimoya growing area in Ecuador is 900 - 1000 mm. Cherimoya is not suited to the lowland tropics and can only be grown at the higher altitudes (Purseglove, 1968). Thus it distributed not only in the tropical highlands but also in warm temperate zones such as Spain, Chile, Peru, and California. It has recently also been introduced into Japan.

### *Arising problems in present cultivation*



**Fig. 1-1-1.** Strong vegetative growth with vertical flushing. Fruit bearing is hard to observe. Pak chong horticultural research station in Thailand.

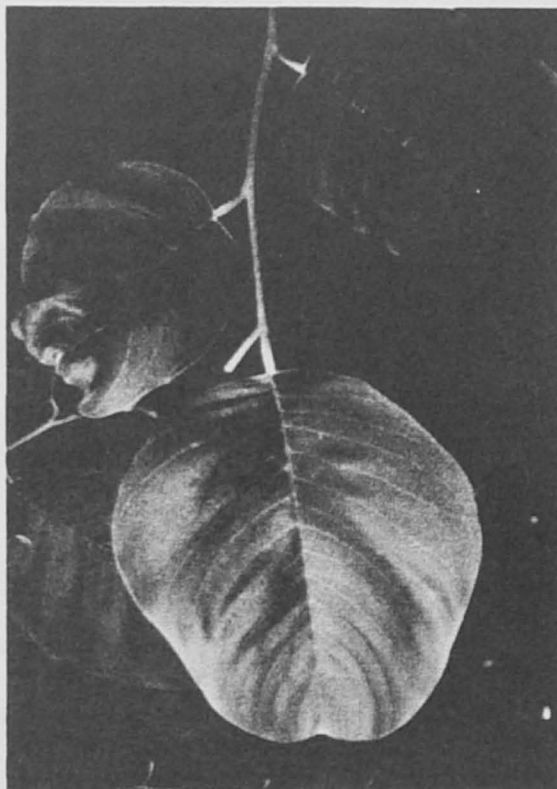
First, limited growth under hot and dry environment is pressing issue. Actually, cherimoya introduction into monsoon Asia has not succeeded due to high temperature. Trial introductions into Thailand highland (Fig. 1-1-1) and Okinawa islands have been observed to produce many strong vertical shoots with poor flowering. High temperature caused leaf shrinking and sun burn (Fig. 1-1-2). These situations were very similar to the observations by Popenoe (1974). He noted that cherimoya trees introduced into Florida, where annual mean temperature is around 25°C, resulted in few asymmetrical fruit. However, the environmental physiology of cherimoya under heat stressed conditions has not been investigated. Hence, little is known about the mechanism of growth suppression or yield reduction under high temperatures.

Second problem is the difficulty of pollination and fruit-set. Cherimoya has clear protogynous dichogamy where the female and male organs mature at different times in the same flower: the stigma is receptive before the pollen is shed in the following day (Schroeder, 1943). Such dichogamy is an out-crossing mechanism (Sedgley and Griffin, 1989). As a result, the fruit-set under natural pollination which is usually mediated by beetles is often poor (Gazit *et al.*, 1982). Generally reliable production of cherimoya needs hand-pollination. However, reproductive organs of cherimoya are very sensitive to environmental factors to lose their availability. Especially, high temperature reduces pollen viability and stigmatic receptivity, as

being accompanied by drought. To improve the efficiency of hand-pollination, the effect of environmental variables concerning with temperature during pollination should be described.

Third issue is that cherimoya tree can not withstand snow and frost. Thus, some protection to frost damage is needed when it is introduced to temperate zones.

Among the problems mentioned above, the very critical problem is heat inhibition. Fruit growing season of cherimoya lies usually during hot season in temperate regions. Thus, heat damage to leaf directly may affect fruit productivity. Moreover, the frost protection facilities such as green-house may cause excess rise in temperature and sometimes promote the heat stress except for winter. It is highly needed to clarify the physiological mechanism on heat inhibition of matter production, flowering, and fruit-set to construct adequate management techniques and to improve the production.



**Fig. 1-1-2.** Heat damage of cherimoya leaves. Leaf shrinking probably caused by high temperature with high light.

### ***Environmental physiology of cherimoya***

All aspects of plant growth and development are influenced directly or indirectly by environmental variables (Schaffer and Andersen, 1994). Better understanding of plant growth responses to environmental variables (i.e. light, temperature, water) is crucial to maximize productivity. Heat stress is related to high light. Light intensity directly affect leaf temperature. Only a small fraction of absorbed irradiance energy by leaf is used in photosynthesis and remainder is transformed into heat which causes stress in plant (Fitter and Hay, 1981). In this study, to investigate the growth response of heat stressed cherimoya tree as affected by environmental variables, a series of experiments as follows was conducted; in subsequent



Chapter 2, cherimoya was compared to other *Annona* species to characterize its growth response to environmental variables; in Chapter 3, matter production ability of heat stressed leaves was evaluated and then the mechanism of reduction in leaf CO<sub>2</sub> assimilation was investigated; and in Chapter 4, the heat inhibition of reproductive growth was investigated throughout the stages from floral initiation to fruit development.

## Growth response of cherimoya seedlings

### Section 1

## Effect of temperature: compared with sugar apple (*A. squamosa* L.)

### INTRODUCTION

A comparative study is effective to characterize plant growth response to environmental variables. Cherimoya and sugar apple are commercially important fruit trees among the genus *Annona*. Cherimoya, believed to be indigenous to highland tropics (Morton, 1987), is cultivated in the high-altitude tropics and warm temperate areas. Sugar apple, the origin of which is unknown (Morton, 1987), is now commonly grown throughout the lowland tropics including semi-arid areas. It is commonly cultivated in tropical South America, dry regions in Australia, tropical Africa, and southern Asia. The cultivation is most extensive in India where the tree is also very common as an escape and the fruit is exceedingly popular in market. The sugar apple tree requires tropical dry climate, and has high drought tolerance. The climatic requirements between cherimoya and sugar apple are apparently different.

Temperature is one of the most important factors affecting fruit tree growth (Berry and Björkman, 1980). The physiological responses to temperature have been studied on many tropical fruit crops such as avocado (Lahav and Trochoulis, 1982), litchi (Menzel and Simpson, 1988a), and passionfruit (Utsunomiya, 1992). George and Nissen (1987) reported temperature effects on growth and dry matter production for atemoya. Relatively little

information is available on other *Annona* fruit trees, although the growing area of cherimoya has now expanded to warm climate areas in the temperate zones. In Japan also, it has recently been produced under plastic green-house conditions. Describing the mechanisms of acclimation to high temperatures is necessary to maximize fruit production.

In this section, the effects of temperature on the growth, dry matter production and CO<sub>2</sub> assimilation of cherimoya were examined as compared to sugar apple seedlings to elucidate the differences in ecological requirements between these two *Annona* fruit trees. The hypothesis here is that vigorous vegetative growth of sugar apple under tropical conditions is attributed to high rate of CO<sub>2</sub> assimilation at high temperature even under increasing leaf vapor pressure deficit condition and low vegetative growth of cherimoya under such conditions is caused by sensitive stomatal conductance.

## **MATERIALS AND METHODS**

Seeds of cherimoya and sugar apple were sown in sand on April 4, 1992. Cherimoya seeds were collected from cv. Big Sister fruit in Japan and sugar apple seeds were obtained from cv. 'Nang' fruit in Thailand. Cherimoya cultivar 'Big Sister' was released in California and is now the most popular in Japan. Sugar apple cultivar 'Nang' is native in Thailand. Two months later, well grown uniform seedlings were selected and transplanted into 2.6 l plastic pots filled with a mixture of sand and loam (1:1, v/v), and grown in a plastic green-house for 2 weeks. On June 26, these plants were transferred to a naturally sun lit glass-house. Two different day/night temperature regimes were maintained in the glass-house; 20/15°C (low) and 30/25°C (high). The duration of the day temperatures was 12 h (6:30-18:30). The plants received 13 h to 14 h of natural daylight. Relative humidity ranged from 50 % in the daytime to 80 % in the nighttime. Adequate irrigation and fertilizer were applied during the treatment. There were eight single plant replications per treatment.

At the beginning of the temperature treatments, stem length and leaf number were determined, and the uppermost unfolded leaf was tagged with a label on each plant. After the tagged leaves had fully expanded, the CO<sub>2</sub> assimilation rate ( $A_c$ ), stomatal conductance ( $g_s$ )

and leaf temperature ( $T_L$ ) were measured by a chamber method with a portable photosynthesis system (LI-6200, Li-cor). At the same time, photosynthetic photon flux density (PPFD) was measured with a quantum sensor attached to the chamber of the system. The atmospheric conditions inside the chamber were adjusted to the same conditions outside. The measurements were performed under various light conditions controlled by covering the chamber with different layers of cheesecloth. These were carried out from 1000 h to 1200 h, after the plants were irrigated sufficiently in the early morning. Leaf to air vapor pressure deficit ( $VPD_L$ ) was calculated from the air and leaf temperature and the relative humidity within the chamber. Correlations between  $A_C$  and PPFD were determined by fitting an exponential curve using least squares. The correlations between climatic factors; PPFD,  $T_L$  and  $VPD_L$  and physiological parameters;  $A_C$  and  $g_s$  were investigated.

Shoot length and leaf number were measured every 2 weeks after temperature treatments started. The treatments lasted for 15 weeks, until October 15 and 9 on cherimoya and sugar apple, respectively. At the end of the treatments, all the plants were removed from the pots and the roots were washed with tap water. The plants were divided into leaves, stems and roots. They were then oven-dried at 70°C for 3 days and their dry weights were determined. Starch concentration of each component was determined by the anthrone sulfuric acid method (Morris, 1948). Effects of temperature were tested for significance using t-tests.

## RESULTS

Temperature effect on the vegetative growth was more profound for sugar apple than for cherimoya (Fig. 2-1-1). Shoot length and leaf number were greater at high temperatures than at low temperatures in both species (Fig. 2-1-2). The growth enhancement in these elements by the higher temperature was less in cherimoya than in sugar apple. In cherimoya, root dry weight obtained at the end of the treatments was greater at low temperatures than at high temperatures, but there was little difference in leaf and stem weights (Table 2-1-1). As a result, the shoot/root ratio at low temperatures was lower than at high temperatures. Sugar apple had much greater dry weights for leaf, stem and root at high temperatures. Especially leaf and stem

dry weights markedly decreased and the shoot/root ratio at low temperatures was also lower. In both species, leaf starch concentration was little affected by temperature, while stem and root had higher concentration at low temperatures (Table 2-1-1). The effect of temperature on the starch concentration of stem and root was greater in sugar apple than in cherimoya.

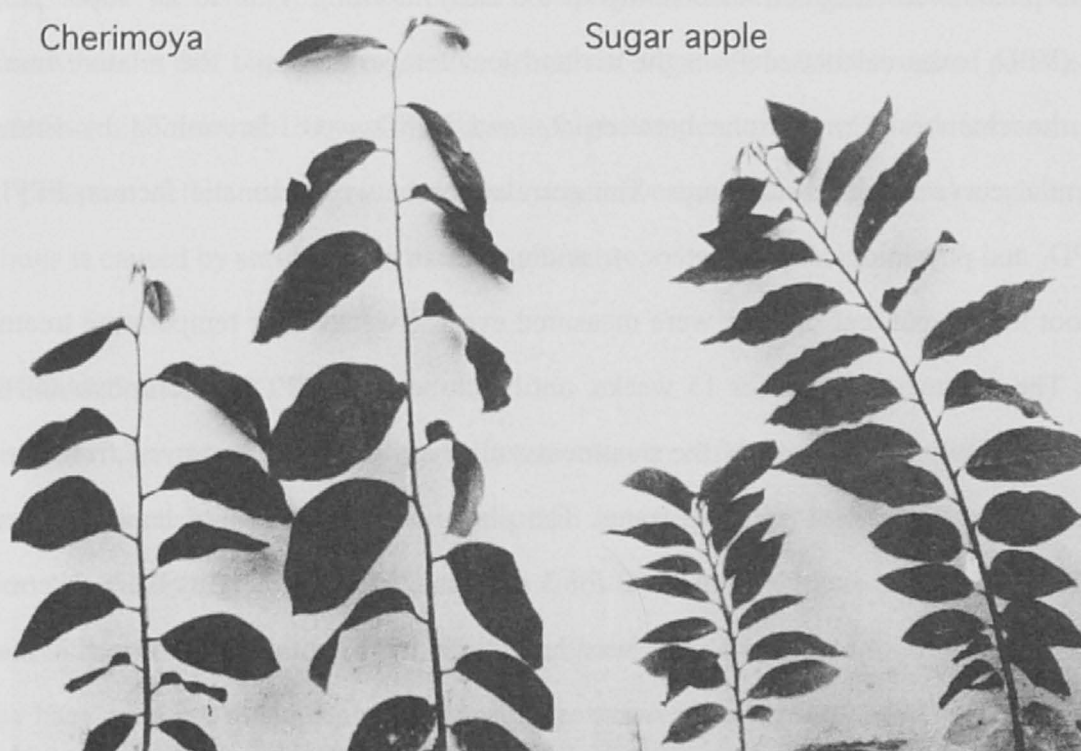
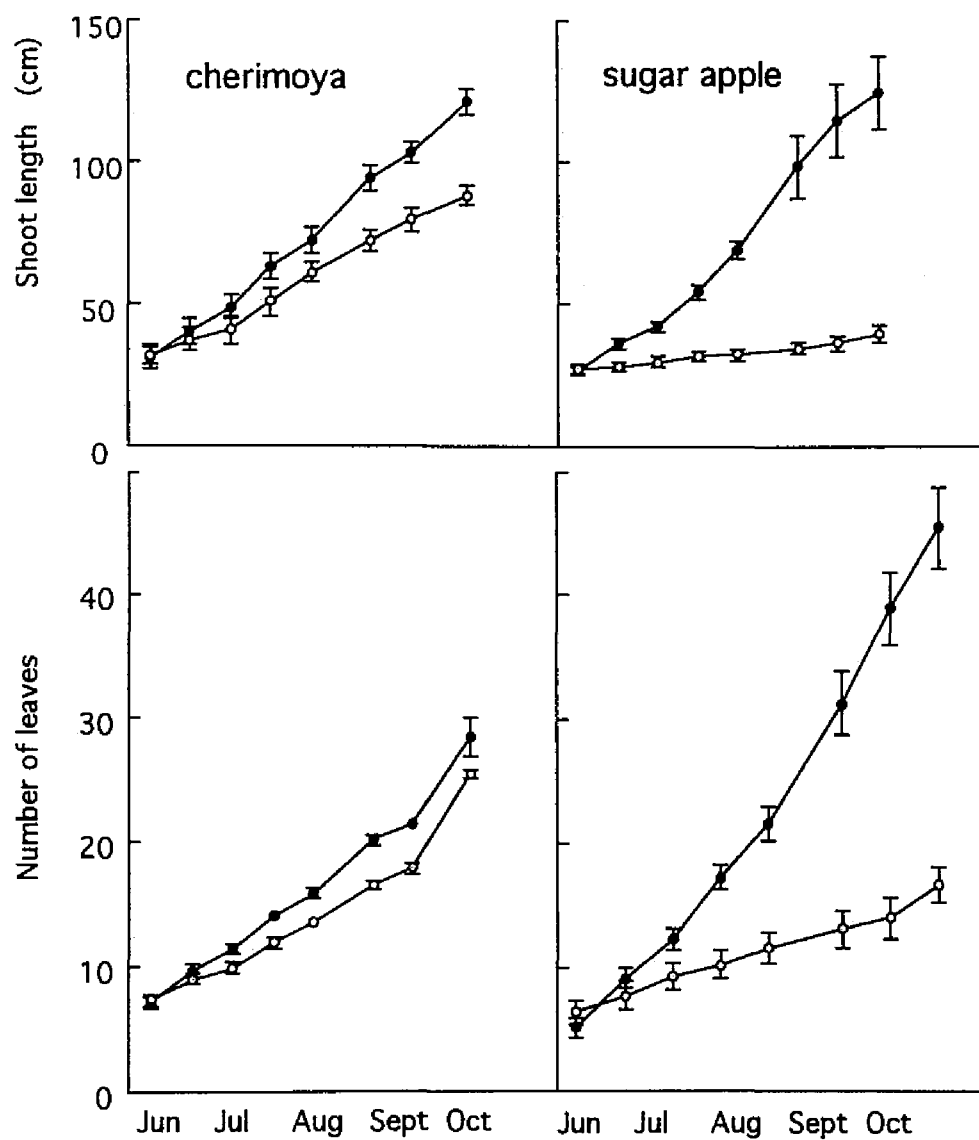


Fig. 2-1-1. Cherimoya and sugar apple seedlings grown at 20/15°C (left) and 30/25°C (right).

In cherimoya, a negative exponential correlation was found between  $A_C$  and PPFD at low temperatures, but  $A_C$  at high temperatures was variable resulting in no exponential relationship between  $A_C$  and PPFD (Fig. 2-1-3). In sugar apple, however, close correlations ( $r^2 = 0.936$  at low and  $r^2 = 0.928$  at high temperatures) between  $A_C$  and PPFD were observed. Above the light saturation point,  $500 \mu \text{mol m}^{-2} \text{s}^{-1}$ ,  $A_C$  of sugar apple at high temperatures was at nearly twice the rate as at low temperatures. The maximum  $A_C$  obtained theoretically from the exponential curve fit was  $14.3$  and  $8.01 \mu \text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$  at high and low temperatures,



**Fig. 2-1-2.** Stem length and leaf number of cherimoya and sugar apple seedlings at 20/15 °C (open) and 30/25 °C (closed). Data are the means of 8 plants. Vertical bars indicate standard error.

respectively.

**Table 2-1-1.** Dry weight and starch content of cherimoya and sugar apple seedlings at different (20/15 °C and 30/25 °C) day/night temperature regimes.

	Temperature (°C)	Dry weight (g)				Starch content (% dw)		
		Leaf	Stem	Root	Shoot/root ratio	Leaf	Stem	Root
Cherimoya	20/15	8.38	7.20	6.12	2.55	4.90	8.83	8.06
	30/25	8.80	8.41	4.88	3.53	5.48	6.26	6.76
	Significance <sup>2</sup>	n.s.	n.s.	*	*	n.s.	*	*
Sugar apple	20/15	1.80	2.09	2.57	1.51	6.58	15.45	14.90
	30/25	7.88	6.40	4.79	2.98	5.68	7.42	9.21
	Significance	**	**	**	*	n.s.	**	*

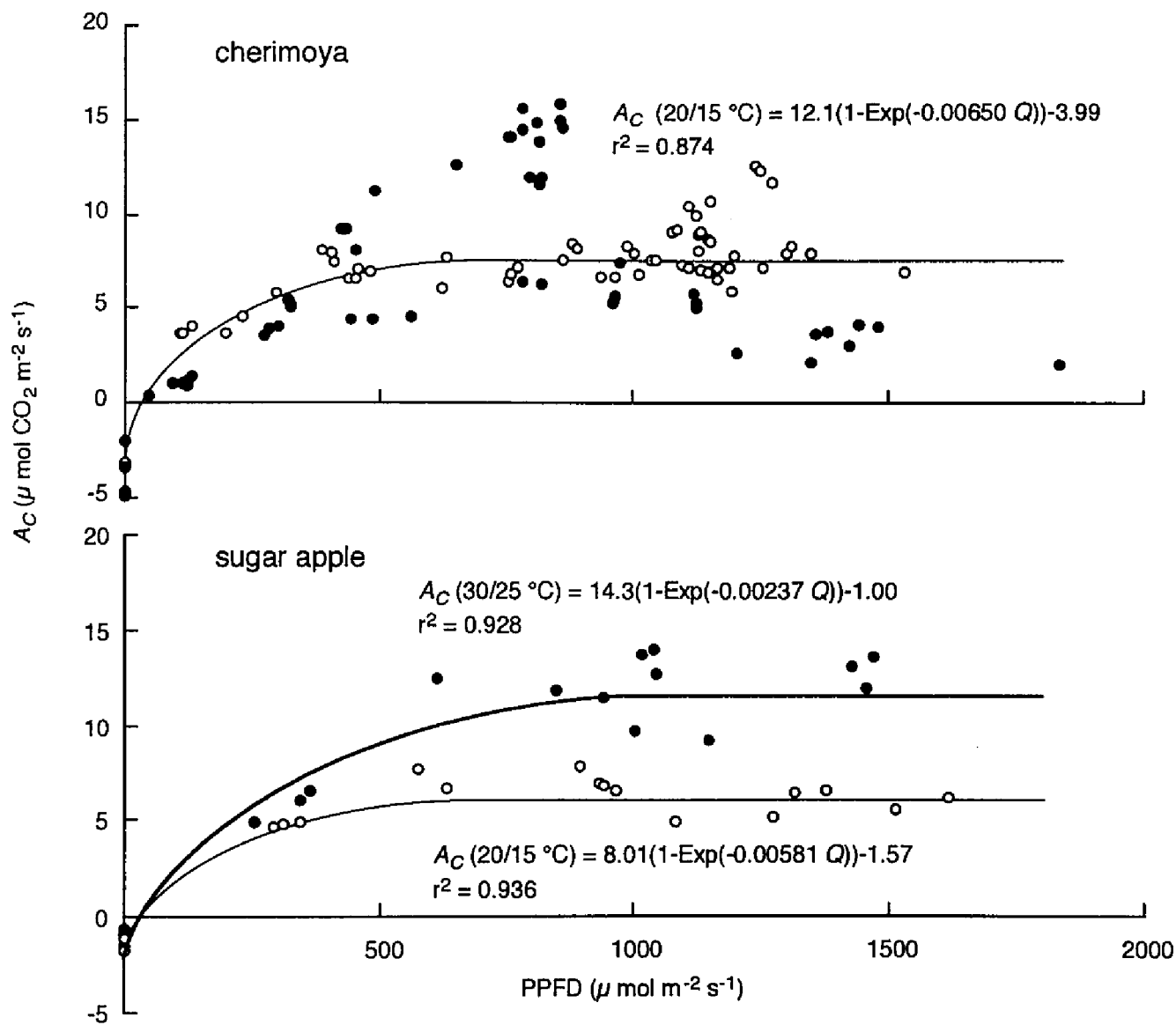
<sup>2</sup>: ns, \*, \*\*: No significant or significant difference at  $p < 0.05$ , and  $p < 0.01$  by t-test, respectively.

The relationship between  $T_L$  and  $A_C$  as affected by  $VPD_L$  is presented in Fig. 2-1-4. Although the minimum  $T_L$  of both species at high temperatures was similar, maximum  $T_L$  of cherimoya was much higher than that of sugar apple. When  $T_L$  was constant,  $A_C$  decreased with an increase of  $VPD_L$ , especially in cherimoya at high temperatures. Under the constant  $VPD_L$ ,  $A_C$  increased with an increase of  $T_L$  in cherimoya and sugar apple, when  $VPD_L$  is lower than 3.5 kPa. In cherimoya,  $VPD_L$  between high and low temperatures varied more widely than in sugar apple.

In cherimoya,  $g_s$  at high temperatures tended to be low as compared with low temperatures (Fig. 2-1-5). In sugar apple, such tendency was not clear since the data points at high temperatures scattered largely. With an increase of  $VPD_L$ ,  $g_s$  decreased under constant  $T_L$  in both species. When  $VPD_L$  was stable,  $g_s$  increased with increasing  $T_L$ . In sugar apple,  $VPD_L$  did not increase up to 4.0 kPa and  $g_s$  did not decrease as markedly as in cherimoya.

## DISCUSSION

The growth response to different day/night temperatures (20/15°C and 30/25°C) varied between cherimoya and sugar apple. Temperature had a direct effect on shoot growth and total dry matter production of sugar apple, but a marginal effect in cherimoya. It is concluded that cherimoya has a wider adaptability to temperature than sugar apple. Thus, high temperature



**Fig. 2-1-3.**  $\text{CO}_2$  assimilation rate ( $A_C$ ) of cherimoya and sugar apple seedlings at 20/15°C (open) and 30/25°C (closed) day/night temperatures as affected by photosynthetic photon flux density (PPFD). Exponential regression curves on 20/15 °C and 30/25 °C are indicated by fine and thick lines, respectively.



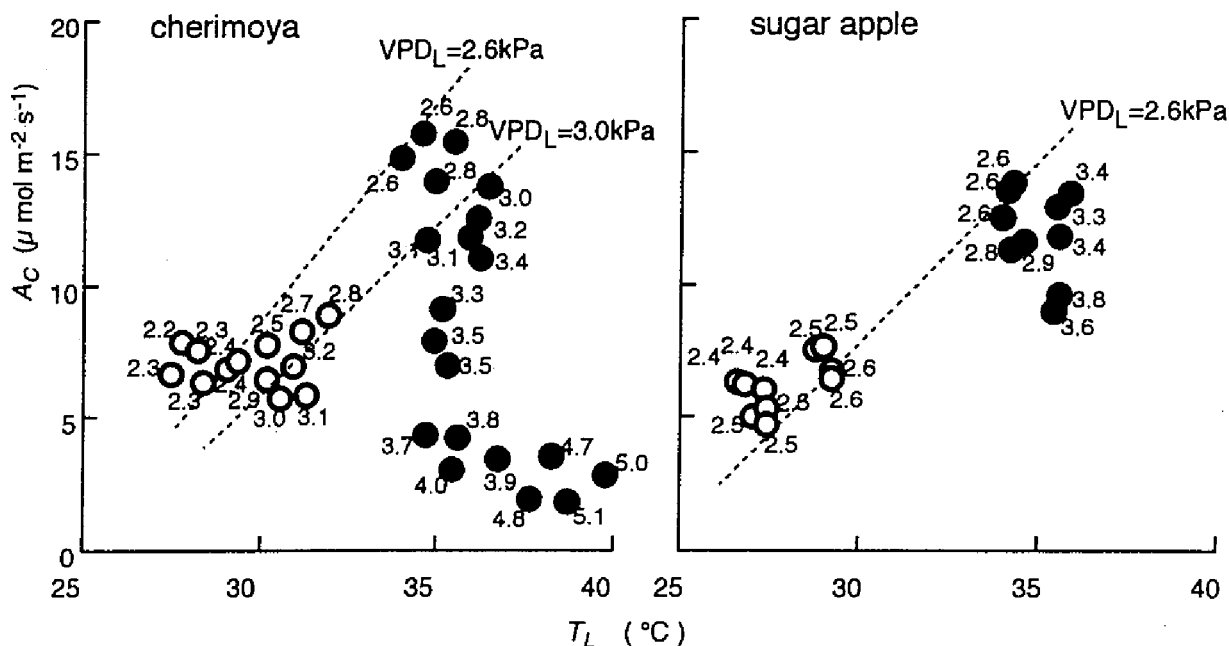


Fig. 2-1-4. Relationship between leaf temperature ( $T_L$ ) and photosynthetic rate ( $A_C$ ) of cherimoya and sugar apple seedlings at 20/15°C (open) and 30/25°C (closed) as affected by leaf vapor pressure deficit ( $VPD_L$ ) above the light saturation point. Values of  $VPD_L$  (kPa) are indicated with the data points. Broken lines are connected with a linear line drawn by eye.

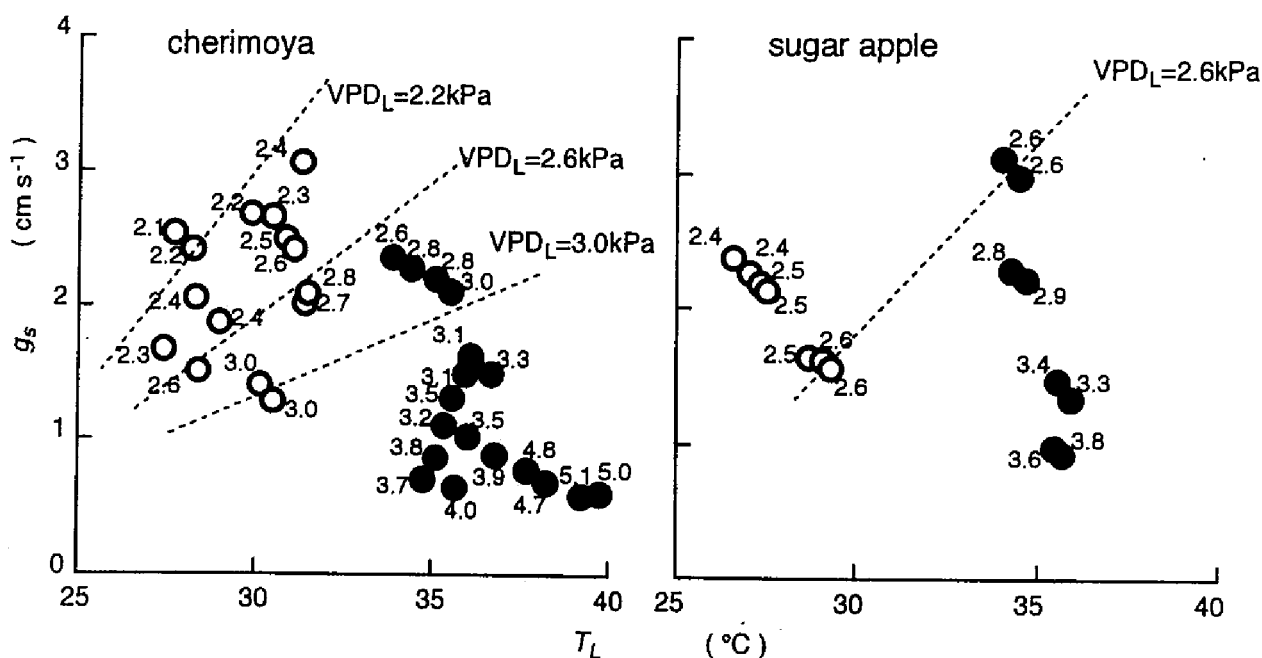


Fig. 2-1-5. Relationship between leaf temperature ( $T_L$ ) and stomatal conductance ( $g_s$ ) of cherimoya and sugar apple seedlings as affected by leaf vapor pressure deficit ( $VPD_L$ ) at 20/15°C (open) and 30/25°C (closed) above the light saturation point. Broken lines are connected with a linear line drawn by eye.

was not expected to substantially affect growth in cherimoya, although it seemed to be slightly more conducive to vegetative growth. Low shoot/root ratio at low temperatures was caused by strong root growth. By contrast, in sugar apple, both shoot growth and dry matter production of all plant parts were seriously suppressed at low temperatures. Low shoot/root ratio at low temperatures was caused by suppressed shoot growth. The total matter production of sugar apple at 30/25°C was about 3 times as that as 20/15°C. This was similar for litchi (Menzel and Paxton, 1985), but the temperature effect on sugar apple was larger than litchi, whose matter production at 30/25°C was about 2 times as that at 20/15°C. This indicates the adaptation of sugar apple to the lowland tropical. Sugar apple does not appear to be acclimatized at 20/15°C conditions.

Effects of temperature on accumulated matter proportion have been reported for many tropical and sub-tropical fruit trees. Increasing temperature from 20°C to 30°C had no effect on dry matter allocation to macadamia root (Trochoulis and Lahav, 1983). In avocado (Lahav and Trochoulis, 1982) and litchi (Menzel and Paxton, 1985) dry matter distribution to root increased at low temperatures. The distribution in mango root also increased significantly with decreasing temperature from 30/25°C to 20/15°C (Whiley *et al.*, 1989), which was similar to the present results with cherimoya. On the other hand, the distribution to sugar apple root increased with the increasing temperature from 20/15°C to 30/25°C. Temperatures ranged from 22/17°C to 32/27°C had little effect on atemoya (a hybrid between cherimoya and sugar apple) stem and root dry matter distribution (George and Nissen, 1987), suggesting that atemoya takes a middle position between cherimoya and sugar apple, reflecting its genetic origin.

Stem and root starch concentration of the two species were higher at low temperatures. A similar response was noted for mango (Whiley *et al.*, 1989) and lychee (Menzel and Simpson, 1995). Low temperature tends to accelerate starch accumulation. Although sugar apple had a higher starch concentration than cherimoya at low temperatures, this resulted from the marked suppression of growth; The total starch content of the whole plant was significantly less than cherimoya at low temperatures. In cherimoya, low temperatures led to the accumulation of

more starch in the root and stem. The implication of reserve carbohydrates in the flowering and productivity has been reported in many fruit trees. Jones *et al.* (1985) and Sharpless and Burkhardt (1954) found that a low concentration of carbohydrates preceded poor flowering in citrus. A direct relationship between the concentration of starch in woody tissues during the rest period and their subsequent fruit yield was also reported with citrus (Goldschmidt and Golomb, 1982), avocado (Scholefield *et al.*, 1985) and mango (Chako and Ananthanarayanan, 1982). However, recent studies pointed out that gross level of carbohydrates in citrus (García-Luis *et al.*, 1995) and starch concentration in lychee (Menzel and Simpson, 1995) could not be a good indicator of flowering.

Photosynthetic response to temperature has been studied for several species among tropical and sub-tropical fruit trees (Schaffer and Anderson, 1994). The present study showed that  $A_C$  performed by sugar apple at high temperatures was twice of that at low temperatures. This fact, along with vigorous shoot growth at high temperatures reveals that sugar apple grows well under tropical lowland climate conditions and these would have to be duplicated in green-houses for good performance. Low  $A_C$  was one of the reasons to restrict shoot growth more seriously at low temperatures. On the other hand, photosynthetic response to temperature was more complex in cherimoya. Although  $A_C$  at high temperatures was higher at  $500-800 \mu \text{mol m}^{-2} \text{s}^{-1}$  than at low temperatures, it decreased greatly when plants were exposed to intensive irradiance above  $1000 \mu \text{mol m}^{-2} \text{s}^{-1}$ . This suggests that cherimoya easily suffers from adverse effects on the photosynthetic process by heat stress and on stomatal limitation by increasing  $\text{VPD}_L$ . Havaux *et al.* (1987) detected that irreversible heat damage to PSII when the temperature exceeded  $37^\circ\text{C}$ . Data shown in Fig. 2-1-4 indicates that  $T_L$  of cherimoya is more prone to increase and may consequently reach about  $40^\circ\text{C}$ . Such an increase in  $T_L$  will involve a substantial reduction of  $A_C$  in cherimoya.

Stomata control  $A_C$  through resistance to the transfer of  $\text{CO}_2$  between leaf intercellular space and ambient air. It is known that stomatal response to temperature is strongly influenced by  $\text{VPD}_L$  (Berry and Björkman, 1980). In the present study, in cherimoya,  $A_C$  decreased with increasing  $\text{VPD}_L$ . Extremely high  $\text{VPD}_L$  brought about suppressed  $A_C$  at high  $T_L$ . In sugar

apple, the effect of  $VPD_L$  was marginal and hence  $A_C$  increased directly with the increase of  $T_L$ . Menzel and Simpson (1986b) noted a direct response of lychee stomata to changes in  $VPD_L$ , and the response was more sensitive at higher temperature. This is similar to the present results for cherimoya. The tendency for high  $VPD_L$  to consequently restrict  $A_C$ , as seen in cherimoya, is consistent with results on citrus (Brakke and Allen, 1995), avocado (Scholefield *et al.*, 1980) and litchi (Menzel and Simpson, 1986b). In sugar apple,  $A_C$  was greater at high temperatures although  $VPD_L$  increased, indicating that temperature directly affected the photosynthetic process to promote its activity.

This study indicates that sugar apple requires warm to hot conditions for optimal growth. This would be supported by the frequent cultivation of sugar apple in lowland and semi-arid tropics, because it seems suitable for sugar apple to grow relatively hot environment even if  $VPD_L$  becomes also high under intensive irradiance. It was also found that cherimoya was susceptible to hot and dry conditions, since increasing  $VPD_L$  with high temperatures and high irradiance decreased photosynthesis. Manipulation of the aerial environment using over-tree misting or shading will be necessary to keep  $VPD_L$  at low level during hot seasons for cherimoya cultivation.

## SUMMARY

This Section is to compare the growth and physiological response of cherimoya and sugar apple seedlings to examine their adaptability to 20/15°C and 30/25°C day/night temperatures in a sunlit glass-house condition. Shoot growth was higher at 30/25°C than at 20/15°C in both species. In sugar apple, temperatures had obviously positive effects on shoot growth: the shoot grew more vigorously at 30/25°C. In cherimoya, the temperature effects were relatively small: differences in growth response at the two temperature regimes were smaller than those in sugar apple. Starch content in sugar apple was also higher at 30/25°C, whereas that in stem and root of cherimoya was higher at 20/15°C. Under low irradiance,  $A_C$  of the two species was higher at 30/25°C than at 20/15°C. In cherimoya,  $A_C$  decreased drastically when the leaf was exposed to high irradiance at 30/25°C. High temperature and irradiance increased the leaf

temperature to above 35°C. With increased leaf temperature, leaf vapor pressure deficit increased and stomatal conductance decreased. This acclimation was associated with the reduction of photosynthesis in cherimoya. Sugar apple had higher photosynthetic activity at 30/25°C, reflected in vigorous shoot growth. Relatively constant leaf temperature in sugar apple seemed to facilitate high rate of  $A_C$  at warm conditions. The results of this study indicate that temperature affected shoot growth and photosynthesis through influencing stomatal conductance *via* leaf vapor pressure deficit in cherimoya.

## Growth response of cherimoya seedlings

### Section 2.

## Effect of irradiance: compared with sugar apple and soursop (*A. muricata* L.)

### INTRODUCTION

Seedling growth responses between cherimoya and sugar apple to temperatures were compared in Chapter 2, Section 1, where wider adaptability to atmospheric temperatures of cherimoya was characterized. The day/night temperatures of 30/25°C were assumed to be over optimum temperature for cherimoya.

Solar radiation determines greatly the plant matter production through photosynthesis. It also affects plant temperature. Plants adapt to change of irradiance level by modifying their morphology and physiological function so that the available light energy is utilized most efficiently. The effect of light intensity on the growth, leaf anatomy and photosynthesis were reported on apple (Barden, 1974; Cripps, 1972), peach (Kappel and Flore, 1983), citrus (Syvertsen and Smith Jr., 1984a), mango (Schaffer and Gaye, 1989b) and carambola (Marler *et al.*, 1994). Tree size, spacing and training system have great effect on light conditions within canopy (Cain, 1971; Jackson, 1978).

However, little is known about the growth response and physiological function to different irradiance level in *Annona* fruit trees. Cherimoya, sugar apple, and soursop are commercially important of fruit-bearing *Annona* trees in sub-tropics and tropics. Cherimoya

favors rather cool and humid conditions. Sugar apple grows well in the areas which have some dry spells in the tropics. Soursop is truly tropical, and prefers hot and humid conditions and plenty of sunlight. It distributed from southern China to Australia and the warm lowland Africa. Recently, cherimoya has been cultivated in the plastic green-house in the warm climate area of Japan. This fruit crop sometimes suffers from leaf sunburn during hot summer probably because of high irradiance and temperature. In this Section, the shoot growth, leaf anatomy and photosynthetic rate under different shade conditions were investigated for young plants of cherimoya, sugar apple and soursop. These informations on growth response and acclimation features to the change of light intensity are useful for determining the tree shade and size and for improving the method of training system.

## **MATERIALS AND METHODS**

On April 4, 1991, seeds of cherimoya (cv. Big Sister), sugar apple and soursop were sowed into sand pot in 1.5 l plastic pots. Cherimoya seeds were collected from the fruits harvested in Wakayama Prefecture. The seeds of sugar apple and soursop were obtained in Thailand but cultivar names were unknown. The seedlings were grown in a greenhouse. On June 1, the uniform seedlings were selected and transplanted into ca. 2.3 l clay pots filled with sand and loam (1:1, v/v). After 2 weeks, these plants were moved outdoors and subjected to four kinds of light levels by placing them in steel frames surrounded by one to several layers of black shade cloth; 100% (C), 45% (S1), 25% (S2), and 5% (S3) of full sunlight (Table 2-2-1). Six plants in cherimoya and 4 plants in sugar apple and soursop were used in each treatment. During the experiment, plants were applied two times with 2g chemical fertilizer (N:P:K=10:10:15).

Just before the start of treatment, the uppermost unfolded leaf was tagged with a label. On Aug. 26, chlorophyll content of the tagged leaf was determined with Green Meter (GM1, Fuji) and expressed as relative value. On the following day,  $A_C$ , stomatal conductance to  $CO_2$  transfer ( $g_c$ ), and transpiration rate ( $E$ ), of the tagged leaf were measured with a portable photosynthesis system (LI-6200, Li-cor). On September 12, the stem length was measured and

the total leaf area was determined with an area meter (LI-3000, Li-cor). Thereafter, all plants were removed from the pots and divided into leaves, stems and roots. They were then oven dried at 70°C for 2 days and their dry weights were measured. The top-root ratio and leaf area ratio (*LAR*) was calculated as leaf + stem dry weight/root dry weight and as total leaf area/dry weight of whole plant, respectively.

**Table 2-2-1.** Irradiance levels on a clear day of treatments during midday hours on August 26 1991.

Treatment	PPFD ( $\mu\text{mol m}^{-2}\text{ s}^{-1}$ )
C (100% sunlight)	1200 - 1800
S1 ( 45% sunlight)	600 - 800
S2 ( 25% sunlight)	250 - 350
S3 ( 5% sunlight)	-90

After the leaf area was determined, a leaf disk (1 cm<sup>2</sup>) was removed from the tagged leaf with a razor blade and immediately weighed to determine the specific leaf weigh (*SLW*). The disk was then fixed in FAA solution. The leaf tissue was dehydrated and embedded in paraffin and sectioned (10  $\mu\text{m}$ ) for measuring the leaf thickness under light microscope.

The experiment was conducted with a completely randomized design. The results of all variables were subjected to analysis of variance. Significance of linear and quadratic models were determined with percent sunlight as the independent variables.

## RESULTS

There was little difference in stem length and plant dry weight between C and S1, but they decreased at heavier shade. (Table 2-2-2). Plant dry weight reduced at S2 and further decreased at S3 for all species. With decreasing light intensity, the top-root ratio tended to decrease in cherimoya but to increase in sugar apple. In soursop the ratio was little affected.

Total leaf area was extremely decreased at S3 in every species (Table 2-2-3). In sugar apple and soursop, the leaf area tended to increase at S1. At S2 the area decreased clearly in



cherimoya and soursop. The *LAR* increased with decreasing light level for all species. Sugar apple and soursop increased the *LAR* more than cherimoya under heavy shade condition. The leaf thickness and *SLW* tended to decrease with increasing the shade level for all species. The degree of reduction in *SLW* was greater in sugar apple and soursop than in cherimoya.

**Table 2-2-2.** Effect of irradiance level on the shoot length, plant dry weight and top-root ratio of cherimoya, sugar apple and soursop seedlings.

Variables	Treatment (sunlight level)				Regression <sup>z</sup>
	C (100%)	S1 (45%)	S2 (25%)	S3 (5%)	
Cherimoya					
Stem length (cm)	29.8	30.4	17.5	5.8	L** r <sup>2</sup> =0.41 Q** r <sup>2</sup> =0.88
Plant dry weight (g)	4.94	5.17	2.03	0.62	L** r <sup>2</sup> =0.58 Q** r <sup>2</sup> =0.84
Top-root ratio	3.79	3.55	3.32	2.45	L** r <sup>2</sup> =0.44 Q** r <sup>2</sup> =0.59
Sugar apple					
Stem length (cm)	18.3	17.5	12	3.5	L, Q ns
Plant dry weight (g)	4.16	3.96	1.98	0.34	L** r <sup>2</sup> =0.64 Q** r <sup>2</sup> =0.86
Top-root ratio	3.16	2.56	4.63	3.86	L* r <sup>2</sup> =0.59
Soursop					
Stem length (cm)	18.3	23	12.5	5	L** r <sup>2</sup> =0.37 Q** r <sup>2</sup> =0.81
Plant dry weight (g)	4.53	4.78	0.99	0.47	L** r <sup>2</sup> =0.67 Q** r <sup>2</sup> =0.82
Top-root ratio	2.94	3.21	3.82	2.12	L, Q ns

<sup>z</sup> L, Q: Linear and quadratic regression, respectively, \*, \*\*:  $P < 0.05$  and  $0.01$ , respectively, ns: not significant.

Shade treatments had little effect on the chlorophyll content in cherimoya (Table 2-2-4). The chlorophyll tended to decrease with decreasing light level in sugar apple and soursop.

In Table 2-2-5,  $A_c$ ,  $g_c$  and  $E$  in leaves grown under different irradiance levels are shown. The effect of light intensity on  $A_c$  was smaller in cherimoya than other two species. There was little difference in  $A_c$  among C, S1 and S2 in cherimoya. The  $A_c$  decreased at S2 in sugar apple and soursop. At S3,  $A_c$  almost reached to the light compensation point in all species. In all species,  $g_c$  tended to increase with decreasing irradiance level except for S3 in sugar apple and soursop. The  $g_c$  was the least at C for all species. In all species,  $E$  was little affected.

**Table 2-2-3.** Effect of irradiance level on the leaf area, leaf area ratio, leaf thickness and specific leaf weight of cherimoya, sugar apple and soursop.

Variables	Treatment (sunlight level)				Regression <sup>z</sup>
	C (100%)	S1 (45%)	S2 (25%)	S3 (5%)	
Cherimoya					
Leaf area (cm <sup>2</sup> )	695.8	698.3	367.5	115.5	L** r <sup>2</sup> =0.56 Q** r <sup>2</sup> =0.86
Leaf area ratio (cm <sup>2</sup> /g)	140.8	135.1	181.1	185.5	L** r <sup>2</sup> =0.58 Q** r <sup>2</sup> =0.86
Leaf thickness (mm)	140	114	92	82	L** r <sup>2</sup> =0.64 Q** r <sup>2</sup> =0.64
Specific leaf weight (mg/cm <sup>2</sup> )	379	365	288	243	L** r <sup>2</sup> =0.40 Q** r <sup>2</sup> =0.82
Sugar apple					
Leaf area (cm <sup>2</sup> )	319.4	368.4	330	69.5	L ns Q** r <sup>2</sup> =0.46
Leaf area ratio (cm <sup>2</sup> /g)	76.7	93.3	166	204	L** r <sup>2</sup> =0.58 Q** r <sup>2</sup> =0.86
Leaf thickness (mm)	76	64	50	50	L** r <sup>2</sup> =0.72 Q** r <sup>2</sup> =0.72
Specific leaf weight (mg/cm <sup>2</sup> )	636	546	336	228	L** r <sup>2</sup> =0.76 Q** r <sup>2</sup> =0.77
Soursop					
Leaf area (cm <sup>2</sup> )	368.3	438.5	163	85.3	L** r <sup>2</sup> =0.41 Q** r <sup>2</sup> =0.67
Leaf area ratio (cm <sup>2</sup> /g)	81	91.8	164.6	181	L** r <sup>2</sup> =0.41 Q** r <sup>2</sup> =0.67
Leaf thickness (mm)	128	112	84	78	L** r <sup>2</sup> =0.74 Q** r <sup>2</sup> =0.76
Specific leaf weight (mg/cm <sup>2</sup> )	586	518	343	282	L** r <sup>2</sup> =0.71 Q** r <sup>2</sup> =0.82

<sup>z</sup> L, Q: Linear and quadratic regression, respectively, \*\*: P<0.05 and 0.01, ns: not significant.

**Table 2-2-4.** Effect of irradiance level on the the chlorophyll content in cherimoya, sugar apple and soursop seedlings.

Variables	Treatment (sunlight level)				Regression <sup>z</sup>
	C (100%)	S1 (45%)	S2 (25%)	S3 (5%)	
Cherimoya	1.36 <sup>y</sup>	1.43	1.43	1.29	L, Q ns
Sugar apple	1.38	1.37	1.21	1.03	L** r <sup>2</sup> =0.53
Soursop	1.74	1.68	1.56	1.39	L** r <sup>2</sup> =0.41

<sup>z</sup> L, Q: Linear and quadratic regression, respectively, \*\*: P<0.05 and 0.01, ns: not significant.

<sup>y</sup>: Reading of Grean Meter.

**Table 2-2-5.** Effect of irradiance level on CO<sub>2</sub> assimilation rate ( $A_c$ ), stomatal conductance to CO<sub>2</sub> ( $g_c$ ) and transpiration rate ( $E$ ) of cherimoya, sugar apple and soursop.

Variables	Treatment (sunlight level)				Regression <sup>z</sup>
	C (100%)	S1 (45%)	S2 (25%)	S3 (5%)	
Cherimoya					
$A_c$ ( $\mu\text{ mol m}^{-2}\text{ s}^{-1}$ )	4.12	4.13	4.75	0.43	Q** $r^2=0.55$
$g_c$ ( $\text{mol m}^{-2}\text{ s}^{-1}$ )	0.11	0.16	0.24	0.26	L** $r^2=0.72$ Q** $r^2=0.73$
$E$ ( $\text{m mol m}^{-2}\text{ s}^{-1}$ )	3.69	3.38	3.66	3.62	L, Q ns
Sugar apple					
$A_c$ ( $\mu\text{ mol m}^{-2}\text{ s}^{-1}$ )	11.26	9.57	6.31	0.28	L** $r^2=0.69$ Q** $r^2=0.92$
$g_c$ ( $\text{mol m}^{-2}\text{ s}^{-1}$ )	0.17	0.31	0.36	0.2	Q** $r^2=0.52$
$E$ ( $\text{m mol m}^{-2}\text{ s}^{-1}$ )	4.04	3.83	3.86	3.58	L, Q ns
Soursop					
$A_c$ ( $\mu\text{ mol m}^{-2}\text{ s}^{-1}$ )	8.93	8.74	6.05	-0.45	L** $r^2=0.55$ Q** $r^2=0.90$
$g_c$ ( $\text{mol m}^{-2}\text{ s}^{-1}$ )	0.17	0.28	0.37	0.22	Q** $r^2=0.50$
$E$ ( $\text{m mol m}^{-2}\text{ s}^{-1}$ )	3.66	3.76	3.69	3.63	L, Q ns

<sup>z</sup> L, Q: Linear and quadratic regression, respectively, \*\*: P<0.05 and 0.01, ns: not significant.

## DISCUSSION

Heavy shade decreases the shoot growth in some kinds of fruit trees. Dry weight of passionfruit plant decreased by 50% at about 30% irradiance level (Menzel and Simpson, 1988b). In apple, dry weights of leaves and stems under 80% shade level were 60% of those under full sunlight (Barden, 1974). In this Section, stem extension growth and plant dry weight were substantially suppressed under lower than 25% of full sunlight in every species. In carambola, however, shoot growth was little affected at 24% of full sunlight (Marler *et al.*, 1994). Thus, growth response of *Annona* seedling seems to be more sensitive to the change of light condition than carambola. This study indicates that higher than 45% sunlight level is required for the normal growth of seedlings in these *Annona* species.

Fruit trees grown under shade condition change their form to adapt the low irradiance. In passionfruit, stem extension growth increased with decreasing light intensity at the sacrifice of leaf area development (Menzel and Simpson, 1988b). On the other hand, peach (Kappel and Flore, 1983) and carambola (Marler *et al.*, 1994) increased the leaf area. However, *Annona* seedlings did not show such morphological change. Both stem length and leaf area decreased with decreasing light level.

Plants commonly respond to shade through increases in top-root ratio and *LAR*. With decrease in irradiance level, only sugar apple tended to increase the top-root ratio although *LAR* increased in all species. The increase of *LAR* was greater in soursop and sugar apple than cherimoya. Thompson *et al.* (1992) reported that the increase in *LAR* was greatest for sun-loving species and least for shade-tolerant species among tropical rain forest trees. Cherimoya may be more shade-tolerant than other two species.

Heavier shade brought about thinner leaves, with lower *SLW* for all species. Similar responses were obtained in citrus (Syvertsen and Smith Jr., 1984a), carambola (Marler *et al.*, 1994) and *Ficus benjamina* (Falis *et al.*, 1982). The leaf grown under shade condition has higher chlorophyll content (Kappel and Flore, 1983; Menzel and Simpson, 1988b; Schaffer and Gaye, 1989b). This is a physiological adaptation to utilize light energy more efficiently. However, the chlorophyll content decreased with increasing shade level in sugar apple and

soursop, and was little affected in cherimoya. These do not suggest that *Annona* seedlings here have such adaptation mechanism.

In citrus (Syvertsen and Smith Jr., 1984b) and chrysanthemum (Holcomb, 1988),  $A_C$  of the leaf decreased under shade conditions. In mango,  $A_C$  decreased with increasing level of shade (Schaffer and Gaye, 1989a). In the present study,  $A_C$  decreased greatly at 5% sunlight level, but the rate at 45% level was comparable to that at full sunlight. This indicates that photosynthetic function is not affected if young *Annona* trees are grown under light levels higher than 45% full sunlight. Under 25% full sunlight,  $A_C$  was a little decrease in sugar apple and soursop, probably because of a reduction of chlorophyll. However,  $A_C$  was little affected in cherimoya. Cherimoya seems to have more effective mechanism to utilize low irradiance compared with other two species.

At 25% sunlight level, plant dry weight decreased although  $A_C$  was not affected in cherimoya. This indicates that the decrease in leaf growth in terms of leaf area and thickness brought about the decrease in total photosynthetic reduction. In soursop and sugar apple, the reduction of dry weight reflected the reduction of both leaf growth and photosynthetic ability. Under 95% shade condition, the inhibition of both  $A_C$  and leaf growth resulted in great decrease of dry weight in every species.

Stomatal conductance was affected by shade in all shade levels than full sunlight. This was different from that obtained in mango, which decreased the conductance under shade condition (Schaffer and Gaye, 1989b). In citrus, changes in  $A_C$  were strongly correlated to stomatal conductance when leaves were exposed to different irradiance level (Syvertsen and Smith Jr., 1984b). The increase in stomatal conductance probably prevented the decrease of photosynthetic ability by increasing  $\text{CO}_2$  flow into leaves in *Annona* fruit trees grown under shade conditions. Little reduction of  $\text{CO}_2$  assimilation capacity at 45% sunlight level seems to be partly caused by greater stomatal conductance. At 5% sunlight level, such compensation effect disappeared because of the inhibitory effect of low irradiance on  $A_C$ .

The present results indicate that the growth of *Annona* fruit trees is greatly reduced under heavier shade condition. These seedlings showed only a little morphological adaptation to the

low irradiance level. Increased stomatal conductance and little reduction of chlorophyll content seems to prevent the decrease in photosynthetic ability under shade condition. The difference in shade tolerance among three species could not be clarified clearly in this study. However, cherimoya seems to be more adaptive to shade compared with sugar apple and soursop.

## SUMMARY

Seedlings of cherimoya (*Annona cherimola* Mill.), sugar apple (*A. squamosa* L.) and soursop (*A. muricata* L.) were grown outdoors at 100 %, 45%, 25%, and 5% of full sunlight level to determine the influence of irradiance level on their growth and photosynthesis. For all species the stem length and dry weight of whole-plant were decreased at 25% sunlight, but there was little difference in them between at 45 and 10% sunlight. Total leaf area tended to increase at 45% sunlight in sugar apple and soursop and to decrease at 25% sunlight in cherimoya and soursop. Leaf area ratio increased with decreasing light intensity in every species, and the degree of increase was the least in cherimoya. With decreasing light intensity, leaves became thinner and the specific leaf weight decreased for all species. Chlorophyll content and photosynthetic rate more decreased at 25% than those at 100 and 45% sunlight in sugar apple and soursop. There were little differences both in the chlorophyll content and photosynthesis among these light intensities in cherimoya. This indicated that cherimoya had higher adaptability to low irradiance than other two species. Under weak light condition the stomatal conductance increased for all species. At 5% sunlight, the plant dry weight was less than about 90% of that of control and photosynthetic rate almost decreased to the compensation point.

## CHAPTER 3

### Photosynthetic response of cherimoya

#### Section 1.

### Effect of temperature

#### INTRODUCTION

The seedling study in Chapter 2 revealed that cherimoya preferred relatively cool condition than sugar apple. The cherimoya seedlings substantially grew better at 20/15°C than 30/25°C day/night temperatures. The result also suggested cherimoya leaves were more sensitive to atmospheric drought condition than sugar apple. However, these results come from seedlings. In this Chapter, trees at fruit-bearing stage were used for more detail study on photosynthetic response of cherimoya.

Temperatures above 30°C increase vegetative growth in some tropical and sub-tropical fruit trees such as litchi (Menzel and Simpson, 1988a), mango (Whiley *et al.*, 1989), and atemoya (George and Nissen, 1987). On the other hand, vegetative or reproductive growth of other sub-tropical fruit trees such as macadamia (Trochoulías and Lahav, 1983), and avocado (Lahav and Trochoulías, 1982) are reduced at high temperatures above 30°C. For cherimoya, however, there has been little research on the growth response to temperature.

Plant growth response is closely related to photosynthesis. Photosynthesis is one of the most heat sensitive processes (Björkman *et al.* 1980). High temperatures increase VPD<sub>L</sub>, and this decreases stomatal conductance to gas exchange for lychee (Menzel and Simpson, 1986a). High temperatures for longan and mango can also damage the internal photosynthetic process

itself and reduce its activity (Yamada *et al.*, 1996). Thus, the heat inhibition against photosynthesis may consist of stomatal limitation caused by increasing VPD<sub>L</sub> (Wilson and Bunce, 1997) and non-stomatal limitation caused by photoinhibition or injury to the Calvin cycle (Ranney and Peet, 1994). Photosynthesis is not always affected only by present temperatures where plants are growing. It can also be affected by previous temperatures when the photosynthetic organs developed. Leaf anatomical morphology was transformable by previous temperatures for carambola (Marler *et al.*, 1994). Although plants differ in their photosynthetic adaptability to temperature as reflected in their native habitats (Ranney and Peet, 1994), the adaptability is affected by leaf acclimatized morphological changes (Marler *et al.*, 1994). A morphologically acclimatized leaf may change its ability to assimilate CO<sub>2</sub>. Meanwhile, neither the morphological leaf acclimation nor the resulting photosynthetic adaptability to high temperatures are well known.

In Chapter 2, it was found that the leaf CO<sub>2</sub> assimilation rate of cherimoya seedlings at 30/25°C day/night temperatures was much lower than at 20/15°C. The results suggested that the difference in the photosynthetic rate was caused by both stomatal and non-stomatal limitations. However, it was not discussed the effect of different leaf morphology developed under variable environments.

The objective of this section is to evaluate photosynthetic ability of leaves grown and developed under different temperature regimes. Furthermore, limited assimilation and decreasing shoot growth rate at high temperatures are discussed.

## **MATERIALS AND METHODS**

### ***Plant materials***

Three-year-old grafted 'Big Sister' cherimoya trees were grown in 10 l plastic pots filled with sand and loam (1:1, v/v). In December 1995, seven trees were transferred to each bay of a sunlit glass-house where day/night temperatures were maintained at 20/15°C (low) and 30/25°C (high). The durations of day temperatures and daylight were 12 h and 12-14 h, respectively. Each plant was irrigated daily and fertilized with 2 g of 10-10-10 (N-P-K)



fertilizer monthly. On March 1, 1996, the trees were thinned, leaving 3 one-year-old stems per tree. The trees were then defoliated and pruned at 5 basal nodes.

### ***Plant growth measurements***

On March 1, 10 one-year-old stems were randomly selected to count the number of newly flushed shoots per stem. Ten of these shoots were then selected at random and each shoot was tagged with a label. The average shoot length, leaf number, and leaf area were measured monthly until July on these tagged shoots. The leaf area was measured with a portable leaf area meter (LI-3000, Li-cor). These shoots were harvested on July 1. After measuring leaf area, they were oven dried at 70°C for 3 days to determine both leaf and stem dry weight. Leaf shoot weight ratio (*LSWR*), specific leaf area (*SLA*), and shoot leaf area ratio (*SLAR*) were calculated from the following equations:

$$LSWR = \text{leaf dry weight/shoot (leaf + stem) dry weight}$$

$$SLA = \text{leaf area/leaf dry weight}$$

$$SLAR = \text{shoot (leaf + stem) dry weight/leaf area.}$$

### ***Leaf chlorophyll content***

Chlorophyll contents of the 2-, 6-, 10-, and 14-week-old leaves were determined. On July 1, the relative values of chlorophyll contents of 20 randomly selected leaves for each temperature regime were first measured with a portable chlorophyll meter (GM1, Fuji). The readings that were obtained by this portable system were then converted into a chlorophyll content on the basis of leaf area using an equation as follows;

$$\text{Chlorophyll (a+b) content (mg dm}^{-2}\text{)} = 2.90 \times \text{Green meter indication} - 1.50$$

This equation was established by the relation between the following two parameters; 1) light absorption by a spectrophotometer (U-1100, Hitachi) at wavelengths of 646 and 663 nm of chlorophyll extract by 80% acetone solution, and 2) the GM1 readings. The determination of chlorophyll concentration from the light absorption was made using the following equation (Schaper and Chacko, 1991):

$$\text{Total chlorophyll content} = 7.18 \times A_{663} + 17.32 \times A_{646}$$

where  $A_{663}$  and  $A_{646}$  are light absorption values at 646 and 663 nm.

### ***Leaf anatomy***

Ten leaves aged 12 weeks were removed for optical microscopic examinations on July 1. The samples were cross sectioned by a microslicer (DTK-1000, D.S.K.) to 25  $\mu\text{m}$  in thickness and stained by safranin solution for morphological observation and measurement of leaf thickness.

### ***Measurement of leaf gas exchange***

Under plant growing conditions at 30/25°C and 20/15°C,  $A_c$ ,  $E$ ,  $g_s$ ,  $\text{VPD}_L$ , PPFD, intercellular  $\text{CO}_2$  partial pressure ( $C_i$ ) and ambient  $\text{CO}_2$  partial pressure ( $C_a$ ) on fully expanded 3 - 4 month old leaves were measured with a portable photosynthetic system (LI-6200, Li-cor) on August 22 (a clear day). The measurements started pre-dawn (0530 h) and continued to after dark (2000 h) at 2-hour intervals. Then, the diurnal changes in  $A_c$ ,  $E$ ,  $g_s$ ,  $\text{VPD}_L$ , and  $C_i$  to  $C_a$  ratio were determined. The correlation between  $1 - C_i / C_a$  at 30/25°C and 20/15°C was calculated and illustrated, to obtain the high to low ratio. The temperature and humidity conditions inside the chamber of the portable system were regulated as closely as possible to glass-house conditions.

Under controlled  $\text{CO}_2$  partial pressure conditions,  $A_c$  and  $C_i$  for 4-month-old leaves developed at 30/25°C and 20/15°C were measured on August 25 from 1200 h to 1300 h with the system described above. The leaves were subjected to high PPFD, 1400 - 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , at an air temperature of 30°C with a  $\text{VPD}_L$  range of 1.4 - 2.5 kPa. In this measurement,  $\text{CO}_2$  was absorbed by soda-lime to operate  $C_a$  inside the chamber so as to be phased out gradually from 35 to 5 Pa in order to regulate  $C_i$ .

## RESULTS

### *Vegetative growth*

New shoots flushed within a week after defoliation on every stem at both temperatures. Shoot length, leaf number and leaf area per single shoot increased more rapidly at 30/25°C than at 20/15°C during the first month after sprouting (Fig. 3-1-1). However, the shoot growth rate decreased gradually at 30/25°C, while at 20/15°C it continued to increase within the first two months. As a result, shoot length, leaf number, and leaf area were greater at 20/15°C than at 30/25°C, 3 months after sprouting.

**Table 3-1-1.** Effects of 20/15°C and 30/25°C day/night temperatures on sprout numbers per stem, dry weight of leaves and stem per sprout shoot, and their total weight of the sprout shoot, leaf stem weight ratio (*LSWR*), specific leaf area (*SLA*), leaf area ratio per shoot (*SLAR*) and leaf thickness.

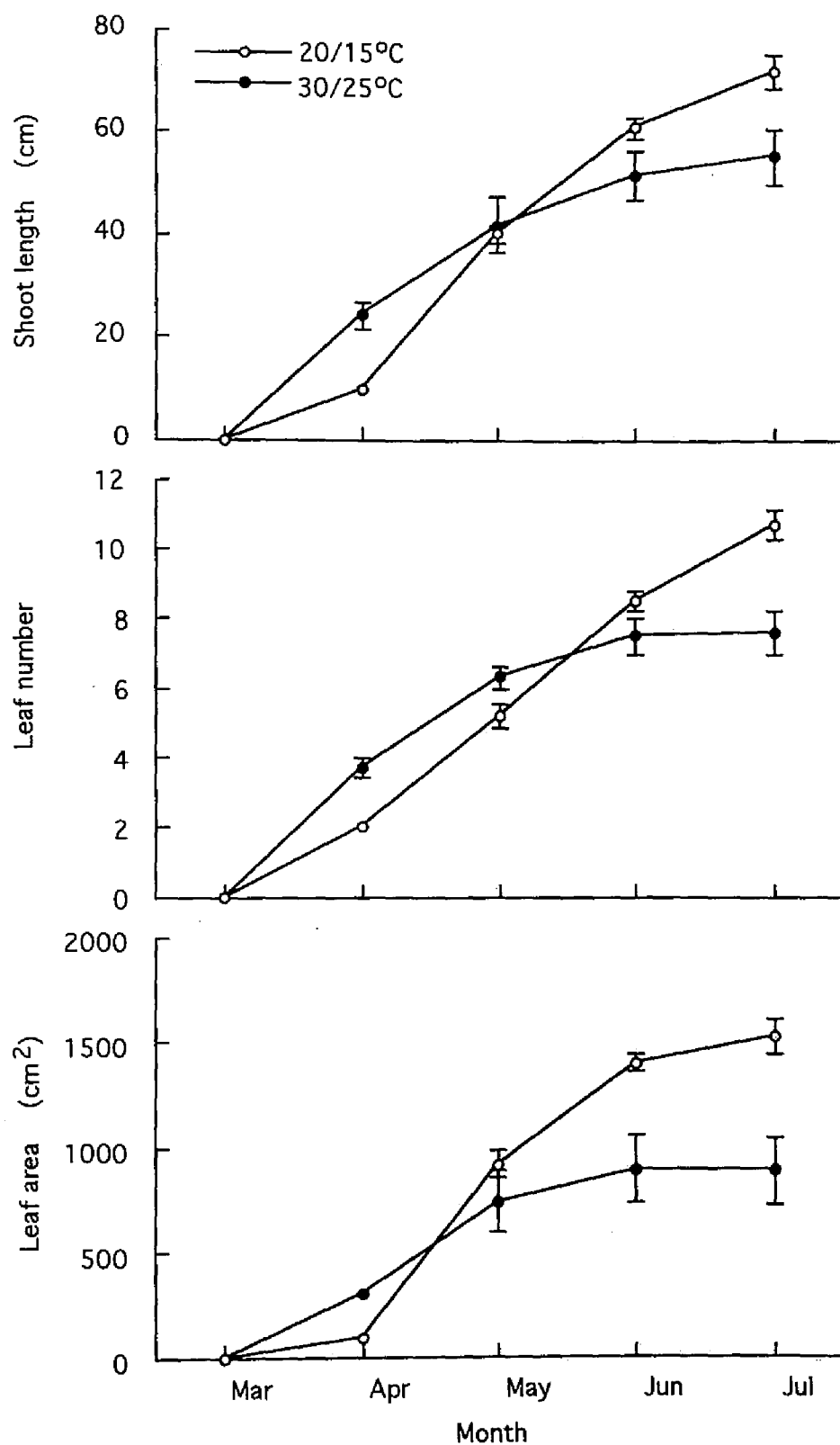
Measurements		Temperature		Significance <sup>x</sup>
		20/15°C	30/25°C	
Number of shoots <sup>z</sup>		2.8	1.9	**
Dry weights (g) of	Leaf	9.84	4.57	***
	Stem	6.51	5.00	ns
	Total (leaf and stem)	16.35	9.57	**
<i>LSWR</i> (g g <sup>-1</sup> )		0.603	0.450	***
<i>SLA</i> (cm <sup>2</sup> g <sup>-1</sup> )		161	239	***
<i>SLAR</i> (cm <sup>2</sup> g <sup>-1</sup> )		96.8	106	ns
Leaf thickness <sup>y</sup> (mm)		186	134	***

<sup>z</sup>: Number of shoots emerged from 1-year-old stem.

<sup>y</sup>: measured with micrometer by light microscope observation.

<sup>x</sup>: ns, \*\*, \*\*\*: Non-significant or significant difference at  $p < 0.01$ ,  $p < 0.001$  by t-test, respectively.

More shoots sprouted at 20/15°C than at 30/25°C (Table 3-1-1). The dry weight of the whole shoot was also greater at 20/15°C. But there was no significant difference in the stem dry weight. The dry matter distribution to the leaves was significantly reduced at 30/25°C. Both *LSWR* and *SLA* decreased at 30/25°C, but not *SLAR*.



**Fig. 3-1-1.** Monthly changes in shoot length, leaf number and leaf area of cherimoya shoot developed at 20/15°C and 30/25°C day/night temperatures. Vertical bars indicate standard errors.

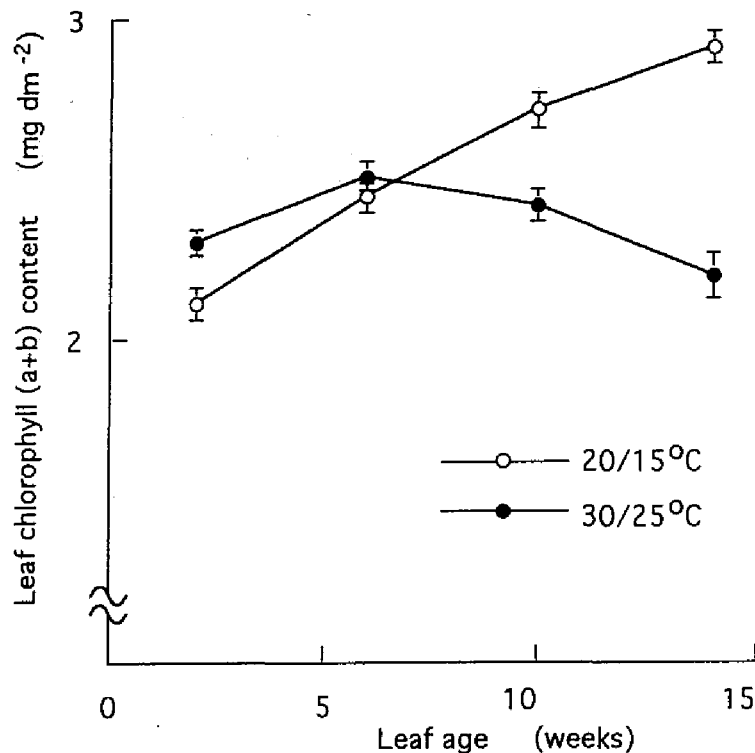


Fig. 3-1-2. Time course changes in chlorophyll content of cherimoya leaves developed at 20/15°C and 30/25°C day/night temperatures. Vertical bars indicate standard errors.

### ***Leaf chlorophyll content***

The chlorophyll content increased with leaf age until 6 weeks after unfolding at both temperatures (Fig. 3-1-2). Thereafter, it decreased at 30/25°C, whereas it continued to increase at 20/15°C. As a result, the chlorophyll content in leaves aged 10 weeks or more was significantly higher at 20/15°C than at 30/25°C.

### ***Leaf anatomy***

The leaves aged 12 weeks at 30/25°C became distinctly thinner than at 20/15°C (Fig. 3-1-3). The leaves grown at 30/25°C had a smaller number of spongy layer cells and thinner spongy and palisade layers. A double layer of palisade cells developed partly in some leaves at 20/15°C.

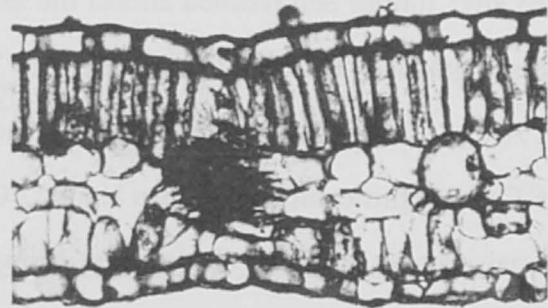
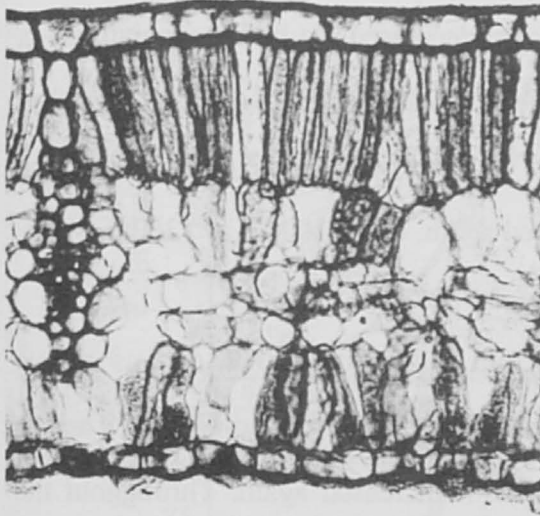


Fig. 3-1-3. Anatomical morphology in cherimoya leaves developed at 30/25°C (right) and 20/15°C (left) day/night temperatures, observed through an optical microscope. Bar indicates 100  $\mu$ m in length.

### ***Leaf gas exchange***

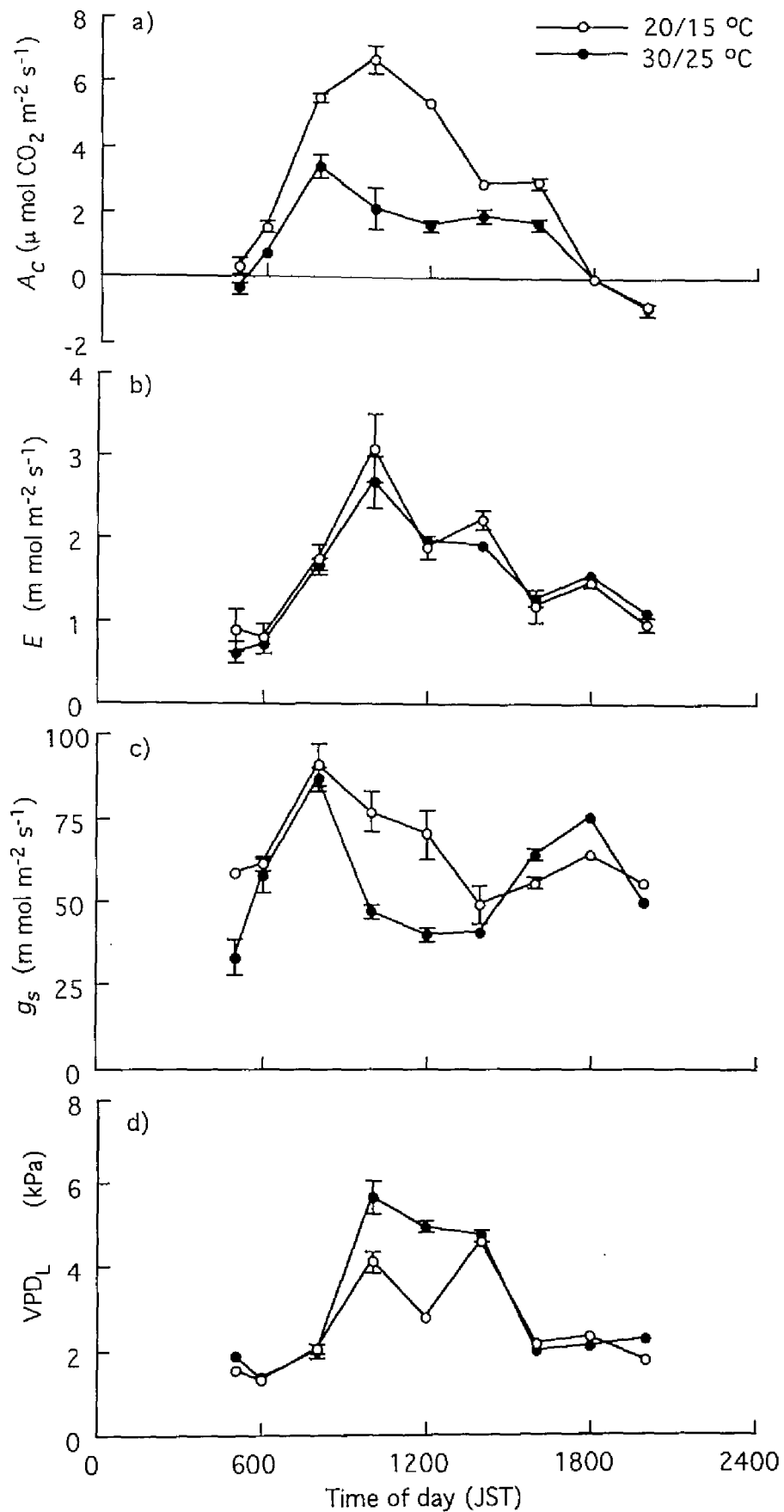
Diurnal changes in  $A_C$ ,  $E$ ,  $g_s$ , and  $VPD_L$  are shown in Fig. 3-1-4. At both temperatures,  $A_C$  increased rapidly just after sunrise (Fig. 3-1-4a). It reached a maximum at 0800 h and 1000 h at 30/25°C and 20/15°C, respectively. Then,  $A_C$  at 30/25°C showed a bimodal pattern with a low second peak at 1400 h. During midday,  $A_C$  at 30/25°C was much smaller than at 20/15°C. The diurnal patterns of  $E$  were very similar at both temperatures (Fig. 3-1-4b). The highest  $E$  was observed at 1000 h, at both temperatures, and then it decreased. At both temperatures,  $g_s$  increased after sunrise and reached almost the same peak at 0800 h (Fig. 3-1-4c). Thereafter,  $g_s$  decreased gradually at 20/15°C and rapidly at 30/25°C. There was a large difference during midday. After 1400 h, at both temperatures,  $g_s$  increased again. From 1000 h to 1200 h,  $VPD_L$  at 20/15°C was significantly lower than at 30/25°C (Fig. 3-1-4d).

Diurnal changes in  $C_i$  to  $C_a$  ratio are shown in Fig. 3-1-5. At 0530 h,  $C_i$  was almost identical to  $C_a$ . After that,  $C_i / C_a$  began to decrease until 1000 h from around 1.0 to 0.8 and to 0.6 at 30/25°C and at 20/15°C, respectively. After that, it increased again. Throughout the daytime, the ratio of  $1 - C_i / C_a$  at 30/25°C to that at 20/15°C was almost constant, 0.586 (Fig. 3-1-6). At both temperatures, linear regression curves between  $A_C$  and  $C_i$  with high correlation coefficients were observed (Fig. 3-1-7). The coefficients of the initial slope of the regressions were 0.038 and 0.058 at 30/25°C and at 20/15°C, respectively.

## **DISCUSSION**

### ***Vegetative growth***

High temperatures have been reported to increase vegetative flushing of some tropical and sub-tropical fruit trees. As discussed in Chapter 2, the growth response to temperature is varied for plant species. In the previous Chapter, shoot growth of cherimoya seedling was accelerated by high temperatures, although the growth promotion was less than sugar apple. George and Nissen (1987) found that atemoya, a hybrid of cherimoya and sugar apple, maintained the highest vegetative growth throughout an 11-week treatment period at 32/17°C day/night temperatures ranging from 17/12°C to 32/17°C. In the present study, cherimoya



**Fig. 3-1-4.** Diurnal changes in a)  $\text{CO}_2$  assimilation rate ( $A_c$ ), b) transpiration rate ( $E$ ), c) stomatal conductance ( $g_s$ ), and d) leaf to air vapor pressure deficit ( $\text{VPD}_L$ ) of cherimoya leaves grown at 20/15 °C and 30/25 °C day/night temperatures. Vertical bars indicate SE.



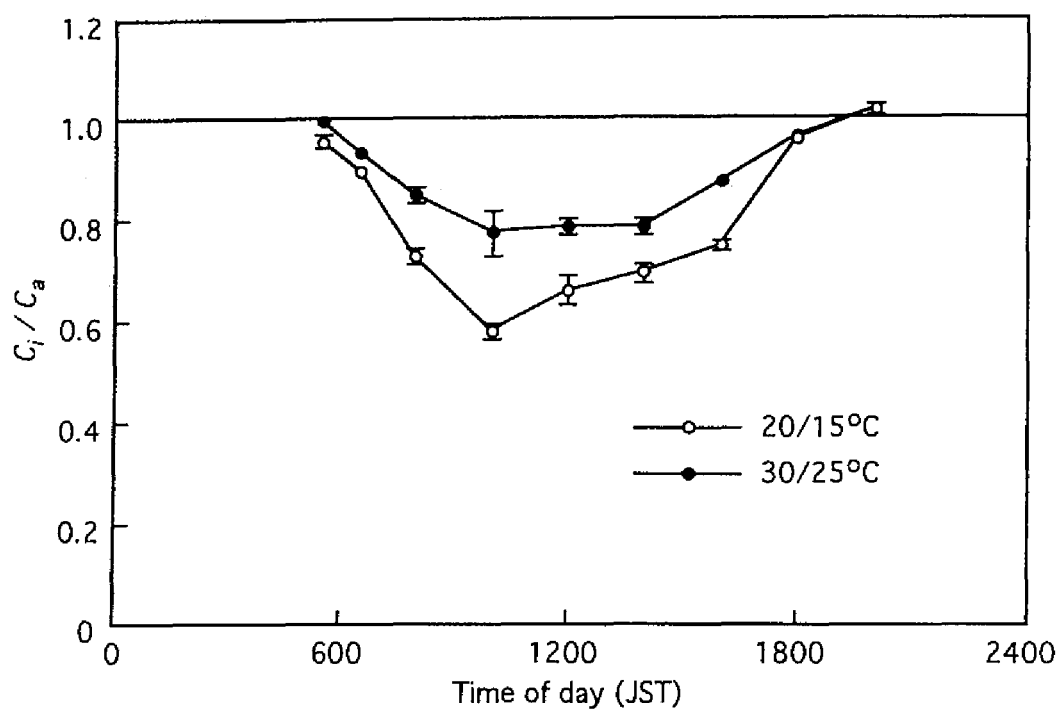


Fig. 3-1-5. Diurnal changes in intercellular to ambient  $\text{CO}_2$  partial pressure ( $C_i/C_a$ ) ratio in cherimoya leaves growing at 20/15 °C and 30/25°C day/night temperatures. Vertical bars indicate SE.

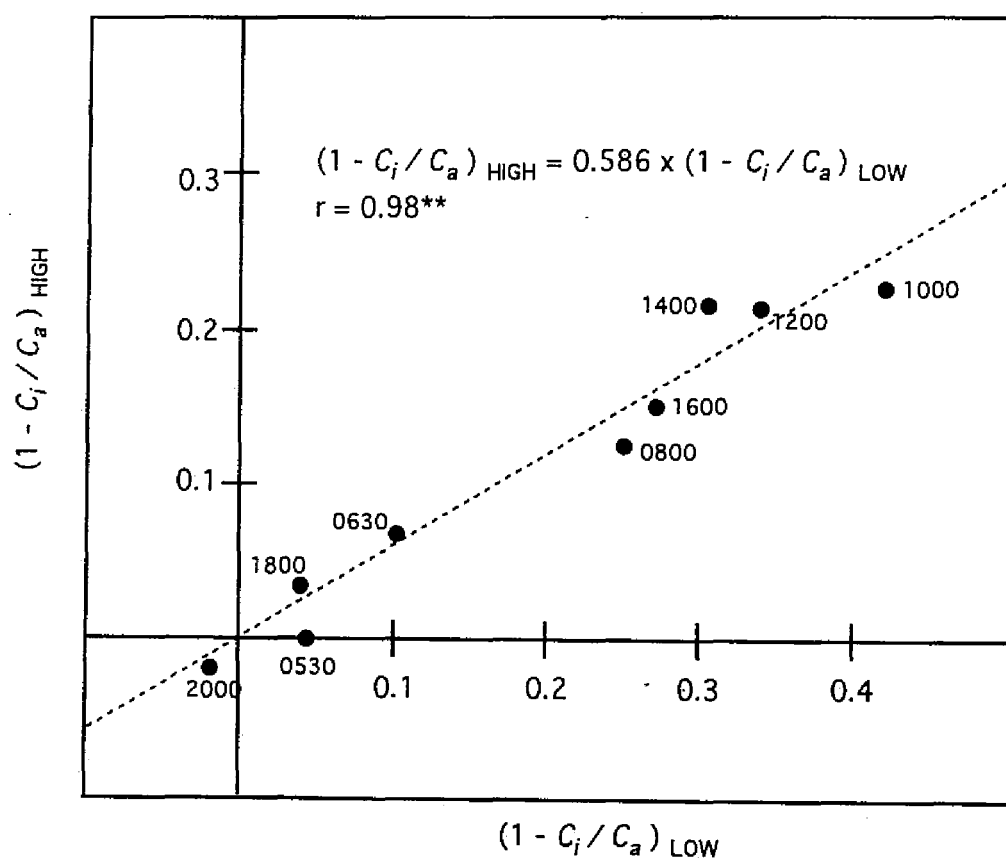
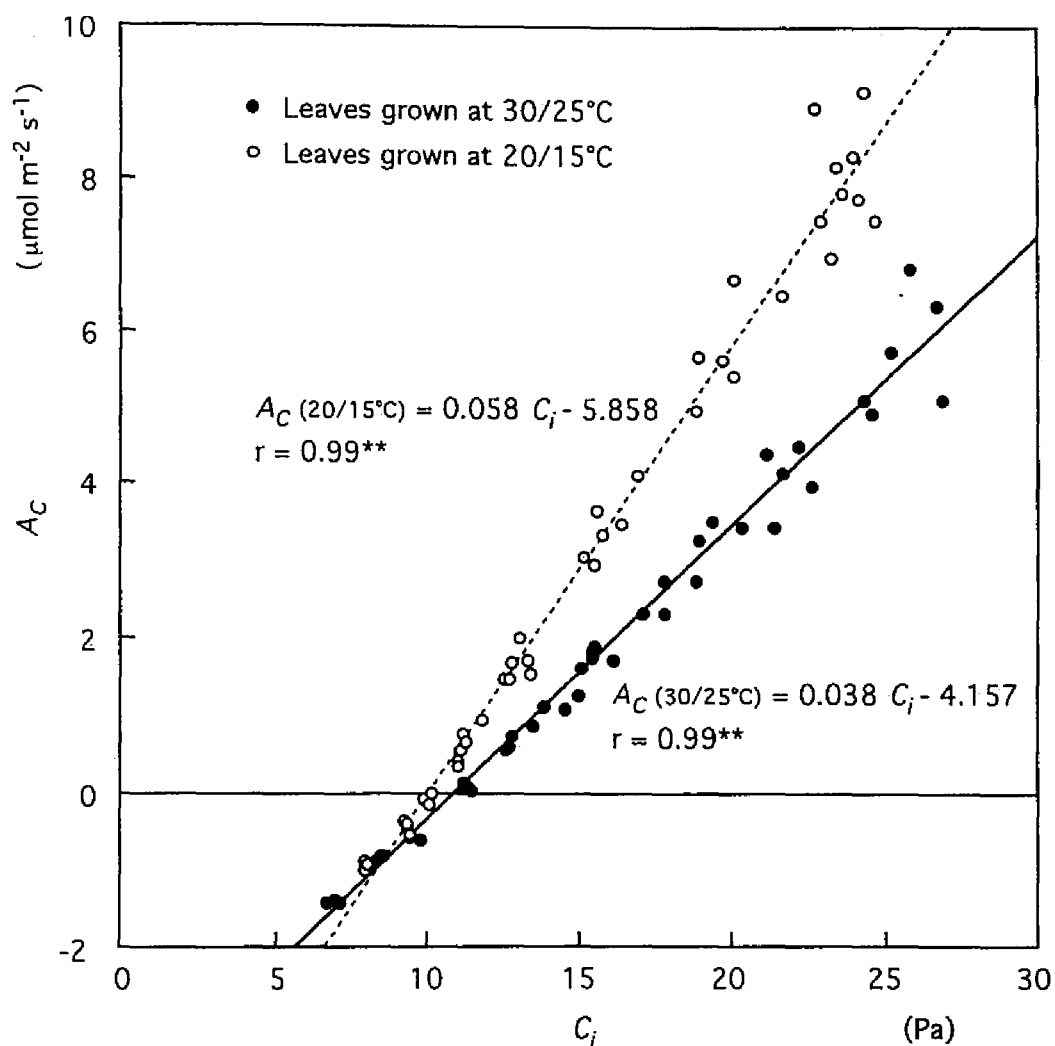


Fig. 3-1-6. Relationship between  $1 - C_i$  (intercellular  $\text{CO}_2$  partial pressure)/ $C_a$  (ambient  $\text{CO}_2$  partial pressure) in cherimoya leaves growing at 20/15 °C (LOW) and 30/25°C (HIGH) day/night temperatures. Numbers aside data plots indicate measurement time. \*\*, Significant at 1% level.



**Fig. 3-1-7.** Relationship between  $\text{CO}_2$  assimilation rate ( $A_C$ ) and intercellular  $\text{CO}_2$  partial pressure ( $C_i$ ) in cherimoya grown at 20/15°C and 30/25°C day/night temperatures. Measurements were conducted at 30°C air temperature, with 1400 - 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density, 1.5 - 2.4 kPa leaf to air vapor pressure deficit. Simple linear regressions fitted to 20/15°C and 30/25°C are indicated by dashed and solid lines, respectively. \*\*, Significant at 1% level.

grew more vigorously at 30/25°C just after sprouting and thereafter the growth rate gradually decreased. This pattern of growth is similar to that of macadamia at high temperatures (Trochoulías and Lahav, 1983). However, the pattern was different from that of seedlings as shown in Fig. 2-1-1 in Chapter 2. This is probably because the temperature requirement for mature tree is lower than seedling. The continuous reduction of the growth rate at high temperatures was related to the decrease of dry matter production. The reduction of leaf dry weight and leaf/shoot weight ratio at 30/25°C indicates that leaf growth was more largely decreased by high temperatures than stem growth. This tendency is different from those previously reported for mango (Whiley *et al.*, 1989), avocado (Lahav and Trochoulías, 1982) and lychee (Menzel and Paxton, 1985), although young seedling of cherimoya exhibited similar growth response to these trees as shown in Chapter 2.

#### ***Leaf chlorophyll content and anatomy***

High temperatures resulted in a thinner leaf with less chlorophyll content. The tendency of the low chlorophyll content in cherimoya leaf grown at high temperatures was contrary to lemon leaf, in which the chlorophyll content was higher at 42/32°C than at 29/21°C (Martin *et al.*, 1995). Low chlorophyll content is known to occur for mango under shaded conditions (Schaffer and Gaye, 1989b) and for mangosteen under high irradiance (Wiebel *et al.*, 1994). Leaf chlorophyll content of cherimoya, however, was affected little by irradiance (Chapter 2). It appears to be influenced more greatly by temperatures than light intensity.

Development of palisade and mesophyll tissues was less at high temperatures. Decreased leaf thickness is known to occur for macadamia above 30°C (Trochoulías and Lahav, 1983) and for atemoya at 32/27°C day/night temperatures (George and Nissen, 1987). Similarly thin leaves were produced for mangosteen grown under shaded condition (Wiebel *et al.*, 1994).

It is assumed that thinner leaves with a low chlorophyll content may represent a disadvantage for the efficient usage of light energy, and they might have a low potential for photosynthetic performance.

## ***Photosynthesis***

Stomata impose a large limitation on CO<sub>2</sub> assimilation rate (Farquhar and Sharkey, 1982). Reduction in  $A_C$  in cherimoya at high temperatures was attributable to both stomatal and non-stomatal limitations.

The midday suppression in  $A_C$  at high temperatures was closely related to the midday suppression in  $g_s$  (Fig. 3-1-4a and 3-1-4c). Here, the stomatal closure was the main cause of reduction in  $A_C$ . High temperatures reduced  $g_s$  in response to increased VPD<sub>L</sub> (Fig. 3-1-4c and 3-1-4d). A similar result was reported for Atemoya that the lowered  $g_s$  at high VPD<sub>L</sub> resulted in limiting midday  $A_C$  (George *et al.*, 1990). At low temperatures,  $A_C$  varied largely due to PPFD, although  $A_C$  reached a peak a little before noon. This result suggests that  $A_C$  was more pronouncedly affected by the irradiance than  $g_s$ , at low temperatures, and that a slight depression of  $A_C$  in the afternoon can be explained by midday water stress, commonly observed even in non-stressed plants (Brakke and Allen, 1995). There was little difference in  $E$  between the two temperatures, despite a significant difference in  $g_s$  at midday, 1000 h to 1400 h (Fig. 3-1-4b and 3-1-4c). This is because a positive effect promoting  $E$  caused by increasing VPD<sub>L</sub> was offset by a negative effect caused by decreasing  $g_s$ . This response was thought to be an effective mechanism to conserve water across wide temperature variations. George *et al.* (1990) reported a similar trend whereby stomatal closure and reduction in  $A_C$  coincided under heat stress in Atemoya. A decrease in  $g_s$ , however, may cause extremely high leaf temperature at high ambient air temperatures. High leaf temperature might lead to an irreversible damage of the photosynthetic apparatus (Berry and Björkman, 1980).

A close correlation between  $A_C$  and  $C_i$  indicates non-stomatal limitation to  $A_C$  (Farquhar and Sharkey, 1982). In cherimoya, lower  $A_C$  at high temperatures coincided with higher intercellular to ambient CO<sub>2</sub> partial pressure ratio ( $C_i / C_a$ ), with almost constant  $C_a$  (Fig. 3-1-4 and 3-1-5). The values of  $C_i / C_a$  during midday hours at low temperatures lay around 0.7, which is known to be common value among C<sub>3</sub> species (Monteith, 1963). Consequently, lower photosynthetic activity at high temperatures was assumed to be caused by non-stomatal

limitation. To estimate the non-stomatal effect on  $A_C$ , irrespective of the effect of  $g_s$ , a parameter of  $1 - C_i / C_a$  was designated.  $A_C$  is given by the following equation, using stomatal conductance to  $\text{CO}_2$  ( $g_c$ ) (Von-Caemmerer and Farquhar, 1981);

$$A_C = g_c (C_a - C_i) / P \quad [\text{Eq. 1}]$$

where  $P$  is the total pressure.  $g_c$  is given simply by  $\kappa g_s$ , where  $\kappa$  is an invariable. Eq. 1 can be rewritten as follows:

$$A_C / g_s = k (1 - C_i / C_a) \quad [\text{Eq. 2}]$$

where  $k$  is  $\kappa / C_a P$ , also an invariable. Thus,  $1 - C_i / C_a$  reflects  $\text{CO}_2$  absorption ability irrespective of  $g_s$  effect. In the present experiment, the ratio of  $1 - C_i / C_a$  at  $30/25^\circ\text{C}$  to that at  $20/15^\circ\text{C}$  remained constant throughout the daytime (Fig. 3-1-6). At high temperatures,  $1 - C_i / C_a$  was 58.6% of that at  $20/15^\circ\text{C}$ . This suggests that leaves growing at  $30/25^\circ\text{C}$  had 58.6% of the  $\text{CO}_2$  absorption ability of those growing at  $20/15^\circ\text{C}$ , irrespective of stomatal factors.

At natural environment,  $C_a$  is generally constant, where  $A_C$  is in negative proportion to  $C_i$ , as shown in Eq. 1. However, under the artificially controlled  $C_a$  conditions (as shown in Fig. 3-1-7),  $A_C$  is in positive proportion to  $C_i$ , providing that  $g_c$  and  $C_a / C_i$  are constant irrespective of  $C_a$ . The equation describing this relation is given by rewriting Eq. 1:

$$A_C = (g_c / P)(C_a / C_i - 1) C_i \quad [\text{Eq. 3}]$$

This equation provides the theoretical support to the following graphically derived equations in Fig. 3-1-7;

$$A_C (20/15^\circ\text{C}) = 0.058 C_i - 5.86 \quad [\text{Eq. 4}]$$

$$A_C (30/25^\circ\text{C}) = 0.038 C_i - 4.57 \quad [\text{Eq. 5}]$$

The constant terms of these equations were caused by internal  $\text{CO}_2$  sources. Plant cell supplies  $\text{CO}_2$  to the intercellular space by photo-respiration and dark-respiration. This supply is independent of stomatal behavior. In the present experiment, light intensity was so strong that the amount of dark-respiration should not be counted. The constant terms of Eq. 4 and 5 are, therefore, considered to indicate the photo-respiration rates.

The initial slope of the  $A_C / C_i$  curve reflects the activity of the ribulose biphosphate carboxylase-oxygenase (Rubisco) in the leaf proportionally under a strong light intensity

above  $1000 \mu \text{mol m}^{-2} \text{s}^{-1}$  and low  $C_i$  conditions (Farquhar and Sharkey, 1982). In this region,  $A_C$  is known to be limited only by the capacity of the Rubisco (Sharkey, 1985). The proportional coefficients of  $C_i$  in Eq. 4 and 5, in the present study, reflect  $\text{CO}_2$  absorption ability of leaf tissues. The coefficient at  $30/25^\circ\text{C}$  was 0.038, which was 66% of that at  $20/15^\circ\text{C}$ , 0.058. This suggests that leaves grown and developed at  $30/25^\circ\text{C}$  has 66% of the Rubisco capacity of that at  $20/15^\circ\text{C}$ , under the momentary high temperature  $30^\circ\text{C}$  condition.

Low photosynthetic activity is closely related to limited plant growth and fruit productivity. This section indicates that high temperature limits photosynthetic ability in cherimoya through less chlorophyll content, a poor leaf structure of mesophyll tissues, stomatal closure, and low Rubisco activity. Consequently, the cherimoya cultivation is not recommended under high temperature conditions during leaf development. Shading will be effective in producing leaves equipped with a high photosynthetic potential, in the areas where the summer season temperature reaches the same level as in the tropics.

## SUMMARY

Shoot growth, leaf morphology, leaf chlorophyll content, and leaf gas exchange were investigated for cherimoya trees grown in a sunlit glass-house where day/night temperatures were kept at  $30/25^\circ\text{C}$  (high) and  $20/15^\circ\text{C}$  (low). The shoot growth at high temperatures was greater than that at low temperatures until 2 months after sprouting. After that, it became less than at low temperatures. Both leaf number and area also decreased at high temperatures within 3 months after sprouting. The thickness of leaf palisade and spongy layers were obviously thinner at high temperatures. Specific leaf area at high temperatures was significantly larger. The chlorophyll content of the leaves at high temperatures also decreased more than 6 weeks after the expansion. High temperatures reduced  $A_C$  because of both stomatal and non-stomatal limitations.  $A_C$  and  $g_s$  were suppressed during the midday hours at high temperatures caused by increased  $\text{VPD}_L$ , suggesting that stomatal closure reduced  $A_C$ . The ratio of  $1 - C_i$  (intercellular  $\text{CO}_2$  partial pressure) /  $C_a$  (ambient  $\text{CO}_2$  partial pressure) at high to that at low temperatures indicated that  $\text{CO}_2$  fixation ability of leaves grown at high

temperatures had 59% of that at low temperatures, irrespective of stomatal effect. The initial slope of the  $A_C / C_i$  curve, which indicates ribulose biphosphate carboxylase-oxygenase (Rubisco) activity, was 67% for leaves developed at high temperatures as opposed to low temperatures.

## Photosynthetic response of cherimoya

### Section 2.

## Effect of irradiance

### INTRODUCTION

Expansion of cherimoya production has been increasing, however, its introduction into monsoon Asia is not successful (George and Nissen, 1992). The warm climate during summer season in the monsoon Asia is not favorable for cherimoya cultivation. It is suggested that limited growth during summer season reflects photosynthetic suppression under high temperatures and strong light intensity. High light is often associated with water deficit condition (Björkman and Powles, 1984) as well as high temperature. The heat inhibition of assimilation at high day/night temperatures was blamed on stomatal closure by increasing atmospheric drought condition, as shown in the former Section. It is also pointed out that the acclimated leaf to high temperatures became thin, being favorable for elevating the cooling function but not always advantageous for assimilation. George *et al.* (1990) documented that the stomatal sensitivity of custard apple could reduce CO<sub>2</sub> assimilation rate at low relative humidity. Thus, reducing the leaf temperature by shading will be efficient for the improvement of limited assimilation.

In Chapter 2, it was noted that the chlorophyll content of cherimoya leaf was slightly affected by shading and that photosynthetic rate was highest at 25% sunlight. Developmental light environment had little effect on leaf chlorophyll content for mangosteen and a higher



chlorophyll content was observed in 20% or 50% shade (Wiebel *et al.*, 1994). In contrast, mango (Schaffer and Gaye, 1989b) and citrus (Syvertsen, 1984) leaves developed under full sunlight exhibited higher photosynthetic rate than those grown under lower light intensities but displayed low leaf chlorophyll content. However, there is no study indicating optimum shading level for cherimoya trees under the orchard condition. Thus the shading experiment was conducted using mature trees in a orchard. In Japan, commercial cherimoya orchards are usually covered by plastic films to protect frost damage. It is therefore important to evaluate the effect of shading produced by plastic film. Furthermore, the effects of shading fabric were investigated when it is combined with plastic film.

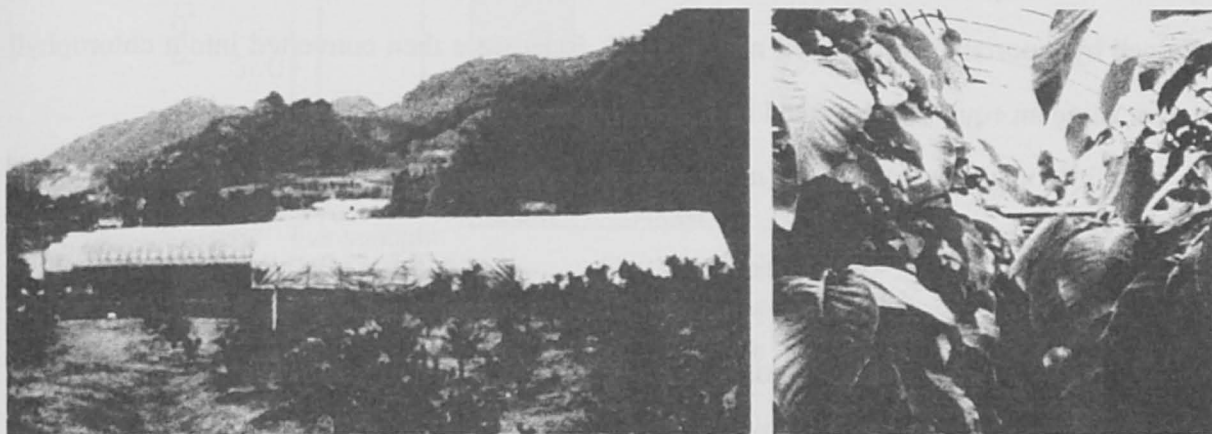
The objective of this Section was to evaluate the effects of developmental shading levels on photosynthetic products, by means of measuring diurnal and midday photosynthetic rate, leaf temperature, leaf to air vapor pressure deficit, leaf water potential, and shoot and leaf morphological acclimation.

## **MATERIALS AND METHODS**

### ***Plant materials and growth conditions***

Six-year-old grafted 'Big Sister' trees were grown under plastic house condition in an experimental orchard of Wakayama Fruit Tree Experimental Station in Japan. On May 17, 1996, the trees were subjected to artificial shade conditions that provide 64%, 24%, and 10% of full sunlight. Shading was prepared under plastic house condition (Fig. 3-2-1). The plastic house (6m x 30m) was divided into 3 sections by white polyethylene fabric partitions. Each section had 4 trees. In light shading condition (64% sunlight), solar radiation was reduced by the plastic film only. Middle (24%) and severe (10%) shadings, which were 37.5% and 15.6% sunlight of light shading conditions, respectively, were given by covering the house with the combination of plastic film and the polyethylene fabric. The bottom halves of each section of the plastic house were left open for air ventilation. The light intensities were measured by a quantum sensor (LI-190SB, Li-Cor) connected to a data logger (21X, Campbell). Air temperature and relative humidity under the canopy at 1.5 m height, for each shading

condition inside the plastic house, were monitored by thermo-hygrometer (CDR-TH1, Sekisui). Under-tree irrigation was conducted by sprinklers weekly to maintain soil moisture to be  $< pF\ 2.4$  at 30 cm depth under ground by monitoring with a tensiometer.



**Fig. 3-2-1.** The outside (left) and inside (right) pictures of shading house, where 64%, 24%, and 10% of sunlight conditions were provided.

### ***Shoot growth, leaf morphology and leaf chlorophyll content***

At the start of shading treatment, laterally extended 10 shoots for each shading level were tagged at the basal point of the terminal leaf. These shoots were produced at about 1.5m above ground level and all these were fruit-bearing at the basal point. To determine shoot growth and leaf morphological characteristics, all the tagged shoots were removed from trees at the tagged positions on October 2. The shoot length, stem diameter and leaf number of the removed shoot were determined, and inter-node length was calculated. Leaf area was measured by a portable leaf area meter (LI-3000, Li-Cor). Thereafter, the sampled shoots were divided into leaves and stems, and then they were dried in a draft-oven at 70°C for 3 days to obtain a constant dry weight. After the measurements of leaf and stem dry weight per a shoot, specific stem length (*SSL*) and specific leaf area (*SLA*) were calculated as shoot length and as total leaf area per unit dry weight, respectively.

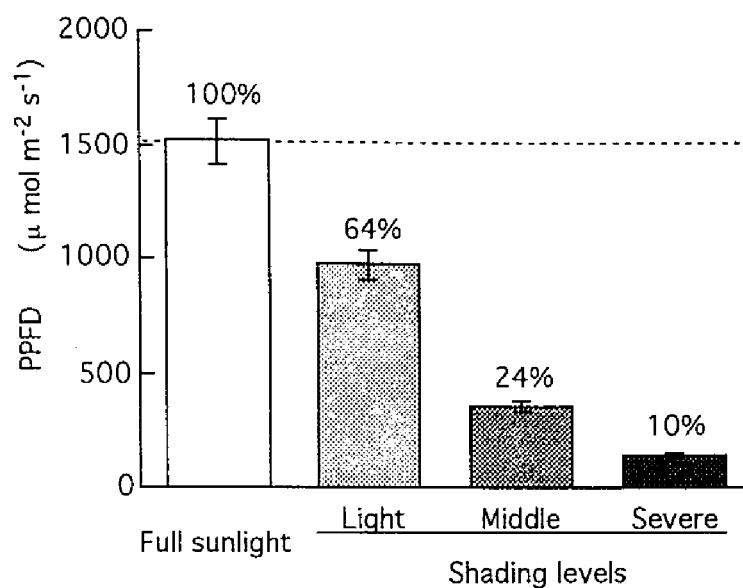
Twenty weeks after growing under the shading conditions, relative values of chlorophyll contents were determined for leaves which were developed during shading, and those had been produced before shading. The pre-shade and post-shade leaves were selected randomly from those proximal and distal to the tagged position. Ten leaves each was used for replications for pre- and post-shade at different shading conditions. The relative values obtained by a portable chlorophyll meter (GM1, Fuji) were then converted into a chlorophyll content using an equation described in the former section;

$$\text{Chlorophyll (a+b) content (mg dm}^{-2}\text{)} = 2.90 \times \text{GM1 reading} - 1.50$$

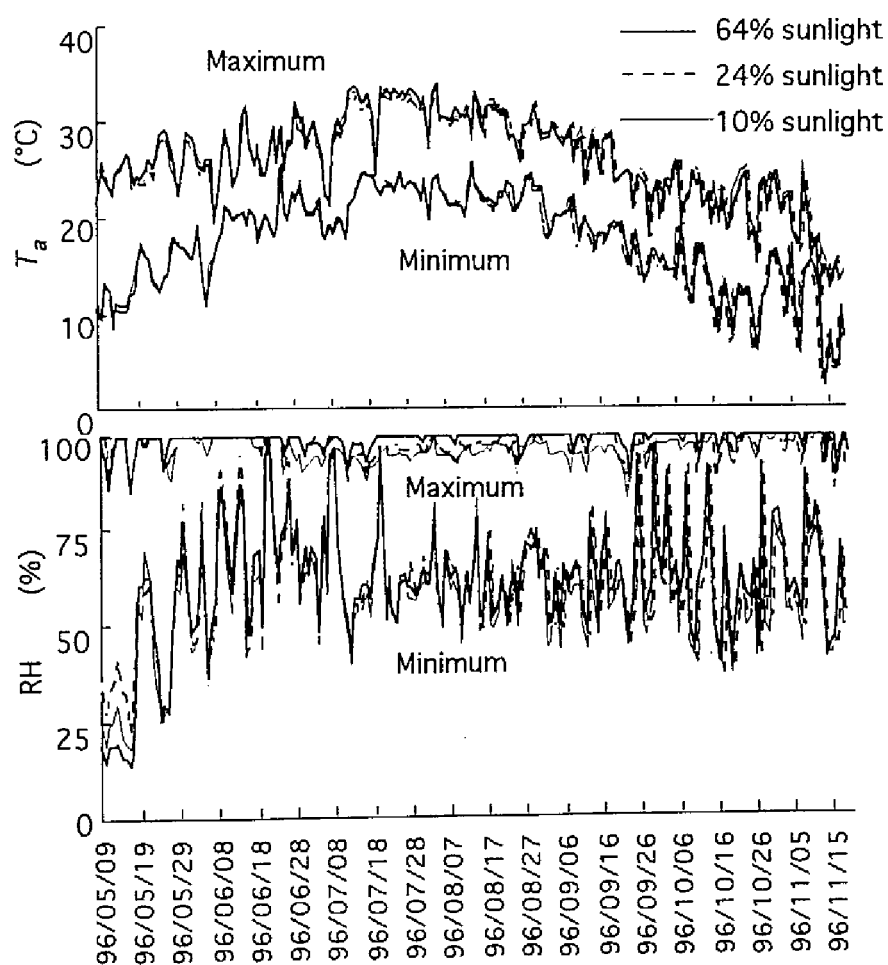
### ***Leaf gas exchange***

Photosynthetic photon flux density (PPFD), air temperature ( $T_a$ ), leaf temperature ( $T_L$ ), leaf to air vapor pressure deficit ( $VPD_L$ ), transpiration rate ( $E$ ), stomatal conductance ( $g_s$ ), and  $A_C$ , were measured for post-shaded 8-week-old sunny leaves on the tagged shoots at 1.5m above ground level grown under the shading house, using a portable photosynthetic system (LI-6200, Li-Cor) during midday hours (1100 h - 1300 h) on June 15, 1996. Similar measurements were performed for leaves under full sunlight. The full sunlight condition was achieved by removing glass from a glass-house aside the plastic house where 4 four-year-old trees had been receiving 88% of full sun. Each measurement was repeated 10 times. The atmospheric conditions inside the chamber of the portable system were regulated as closely as possible to the plant growing conditions. Afterwards, relationship between  $A_C$  and PPFD was established.

Diurnal changes of  $A_C$ ,  $g_s$ ,  $E$ , water use efficiency (WUE) calculated as  $A_C / E$ , PPFD, relative humidity (RH),  $VPD_L$  and  $T_L$  were determined for leaves grown under the 3 different shading conditions, by the portable system mentioned above, on July 13 (a clear day). The measurements were started pre-dawn and continued to after sunset, at about 2-hour intervals. The measured leaves were selected from sunny post-shaded fully mature leaves (over 10 replicates) aged about 8 weeks on a lateral oriented shoot at 1.5m above ground level. The air conditions inside the chamber were controlled so as to follow the ambient condition of each



**Fig. 3-2-2.** Photosynthetic photon flux densities (PPFD) of different shading levels as compared to full sunlight intensities on June 15 1996.



**Fig. 3-2-3.** Daily maximum and minimum air temperature and humidity under various levels of shading throughout the experimental period.

light environment. And then, the relationships between  $A_C$  and  $g_s$ , and stomatal resistance ( $r_s$ , inverse of  $g_s$ ) and  $VPD_L$  during the midday hours (1000 h - 1400 h) were determined.

### ***Leaf water potential***

Diurnal changes of leaf water potential was measured for leaves grown at the different shading levels as described above, using a thermocouple psychrometer (SC10A, Decagon), on August 31. The measurements were started pre-dawn and were continued to after sunset, at about 2-hour intervals. The selected leaves were sunny leaves produced a few weeks after shading was started. Leaves were cut off the trees of each shading level and immediately put into the psychrometer chamber. After leaving sample leaves inside the chamber for 3 hours to come to thermal equilibrium, water potential was determined by dew point measurements.

## **RESULTS**

Light intensity at different shading levels as compared to full sunlight is shown in Fig. 3-2-2. The levels of PPFD at light, middle, and severe shading conditions were 64%, 24%, and 10% of full sunlight, respectively. Shading had little effect on air temperature ( $T_a$ ) and relative humidity (RH) throughout the experimental period (Fig. 3-2-3).



**Fig. 3-2-4.** Heat damage of cherimoya leaves. A severe leaf sun-burn caused by high temperature with high light.

### ***Shoot growth, leaf morphology and leaf chlorophyll content***

A severe sun-burn was observed on sunny leaves in the glass-house, 88% sunlight condition (Fig. 3-2-4). Shoot length and leaf number at light shading (64% sunlight) were the largest of all the shading conditions (Table 3-2-1). Inter-node length was increased by shading, due to lesser effect on reduction in shoot length than nodal number. Remarkable high specific

**Table 3-2-1.** Leaf and shoot growth and morphological characteristics of cherimoya 'Big Sister' at different shading levels under plastic house conditions. Shading was started on May 17 and measurements were made on October 2. Data are based on newly developed part of selected shoots after the shading started.

Variable	Sunlight (%)		
	64	24	10
Shoot length (cm)	64.8 a	43.5 ab	24.9 b
Leaf number	13.3 a	6.8 b	3.6 b
Inter-node length (cm)	4.84 b	5.88 ab	6.36 a
Stem diameter (mm)	6.65 a	5.86 ab	4.23 b
Leaf area/shoot (cm <sup>2</sup> )	2148 a	1405 ab	873 b
Area/leaf (cm <sup>2</sup> )	170.9 b	198.8 ab	231.2 a
Leaf dry weight (g)	8.68 a	4.27 b	2.16 b
Stem dry weight (g)	3.00 a	2.35 ab	0.48 b
Specific stem length (cm g <sup>-1</sup> )	20.3 a	58.1 a	124.5 b
Specific leaf area (dm <sup>2</sup> g <sup>-1</sup> )	2.77 b	3.31 ab	3.84 a

Different letters in the same row indicate significant difference at  $p < 0.05$ .

**Table 3-2-2.** Leaf chlorophyll content of cherimoya 'Big Sister' at different shading levels under plastic house conditions. Shading was started on May 17 and measurements were made on October 2. Data are means of ten 20-week-old leaves developed just before and after starting of shade.

Leaf chlorophyll (a+b) content (mg dm <sup>-2</sup> )	Sunlight (%)		
	64	24	10
Pre-shade leaf	2.47 c	3.29 b	3.65 a
Post-shade leaf	3.01 b	3.19 a	2.93 b

Different letters in the same row indicate significant difference at  $p < 0.05$ .

stem length at severe shading (10% sunlight) represented slender shoots. Stem diameter grew larger at lighter environment. Leaf and stem dry weights were also higher at light shading. Although both the dry weights were suppressed at the deepest shade (10 % sunlight), stem dry weight was less affected by decreased irradiance than leaf dry weight.

The thinner and larger leaves developed under the heavier shade environments. Single leaf area was increased by heavier shading, although the total leaf area per shoot was decreased. Specific leaf area became larger as shading level increased, indicating that leaves at heavier shade reduced leaf thickness.

Leaf chlorophyll content was increased by low light intensity for pre-shade leaves (Table 3-2-2). However, as for that of post-shade leaves, the highest value was observed at intermediate shading (24% sunlight).

### ***Leaf gas exchange***

Midday  $A_C$ ,  $E$ , and  $g_s$  were higher for leaves at 64% and 24% sunlight than the other irradiance levels (100% and 10%), and there were non-significant differences between the light and the middle shading conditions (Fig. 3-2-5). Leaves under full sunlight indicated higher  $A_C$  than at 10% sunlight, but exhibited lower  $g_s$ . At full and 10% sunlight,  $E$  was similar. The shading level linearly affected  $T_L$  and  $VPD_L$ : the highest  $T_L$  and  $VPD_L$  were given for full sunlight.

Under the conditions where the PPFD ranged below  $500 \mu \text{mol m}^{-2} \text{s}^{-1}$ ,  $A_C$  increased with light intensity, and it decreased when PPFD increased more than that level (Fig. 3-2-6).

Diurnal changes of environmental variables and leaf gas exchange responses were shown in Fig. 3-2-7. Generally,  $A_C$  was higher at the lighter environment except for during midday when difference in  $A_C$  between 64% and 24% sunlight was very small. At all the shading conditions,  $A_C$  was relatively higher in the morning. This tendency was pronounced for the lighter environment. Under every shading condition,  $g_s$  tended to decrease generally during the daytime, although  $g_s$  at 24% sunlight indicated substantial increase in the late morning. Although the diurnal patterns of  $E$  of all treatments were similar to those of  $A_C$ , differences

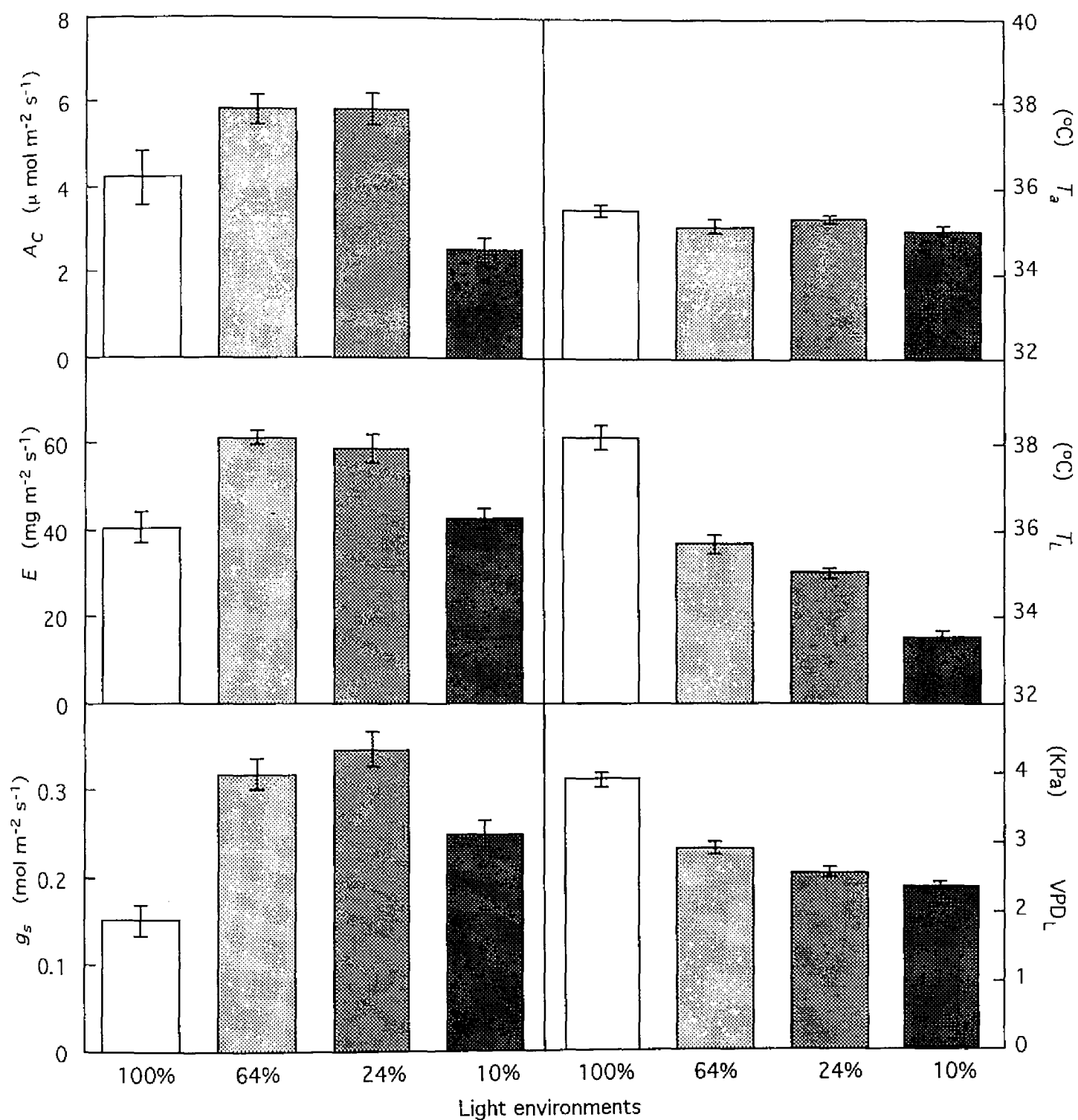
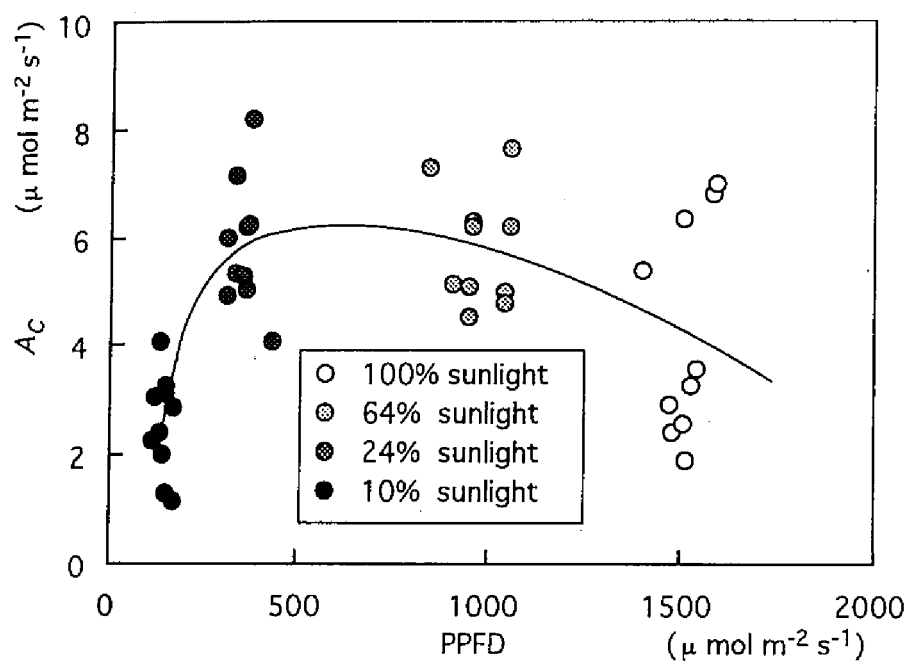


Fig. 3-2-5. Leaf  $\text{CO}_2$  assimilation rate ( $A_C$ ), transpiration rate ( $E$ ), and stomatal conductance ( $g_s$ ), air temperature ( $T_a$ ), leaf temperature ( $T_L$ ), leaf to air vapor pressure deficit ( $\text{VPD}_L$ ) of cherimoya leaves under full sunlight and shaded (64%, 24%, and 10% sunlight) conditions. Measurements were made during midday hours (1100 h - 1300 h) on June 15 1996.





**Fig. 3-2-6.** Relationship between leaf  $\text{CO}_2$  assimilation rate ( $A_C$ ) and photosynthetic photon flux density (PPFD) of cherimoya leaves under different light environments measured during midday hours (1100 h - 1300 h) on June 15 1996. The solid line indicates a quadratic regression curve  $A_C = -5\text{E}^{-6} \text{PPFD}^2 + 0.0095 \text{PPFD} + 2.0404$  which has an  $r = 0.57$  and is significant at  $F < 0.01$ .

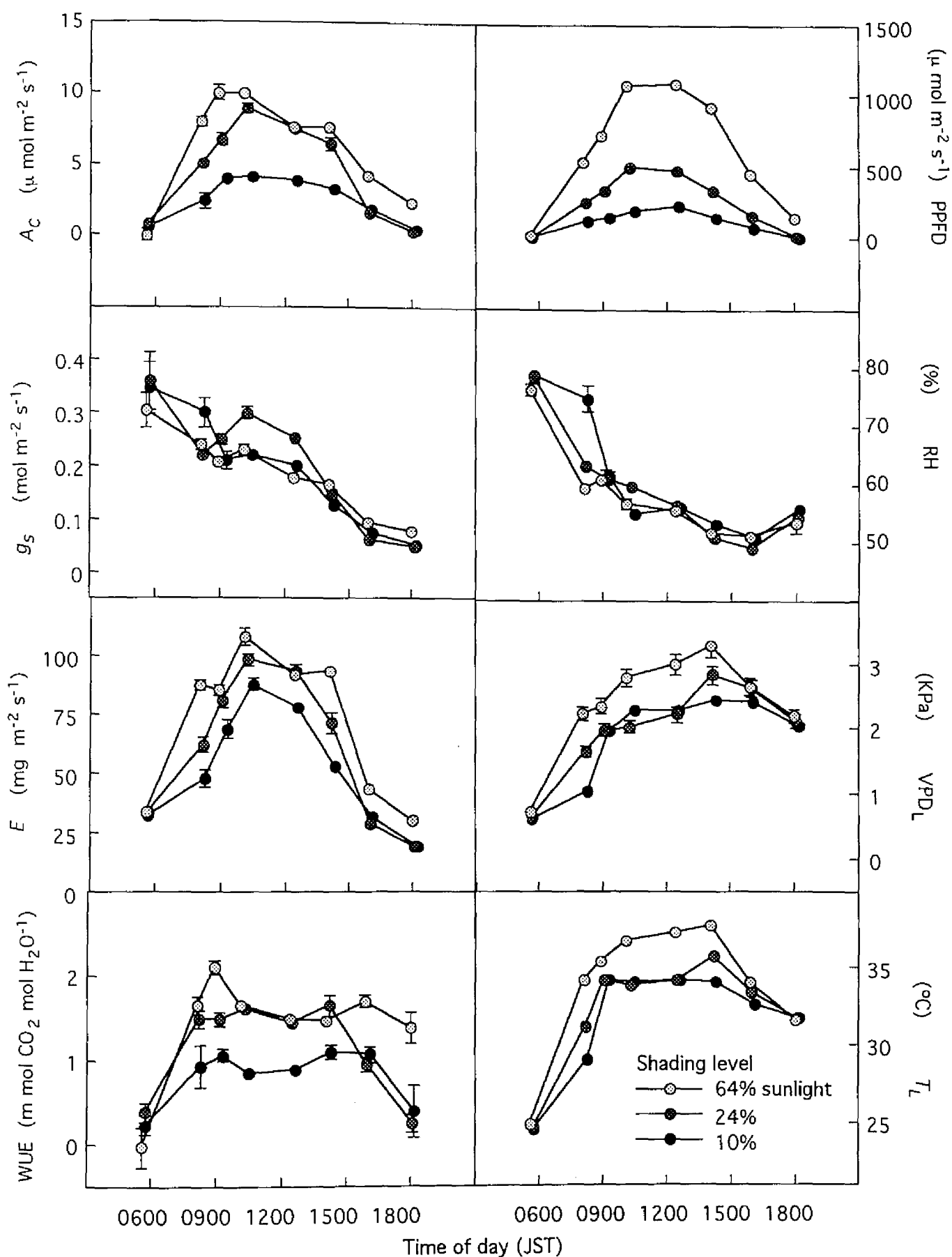
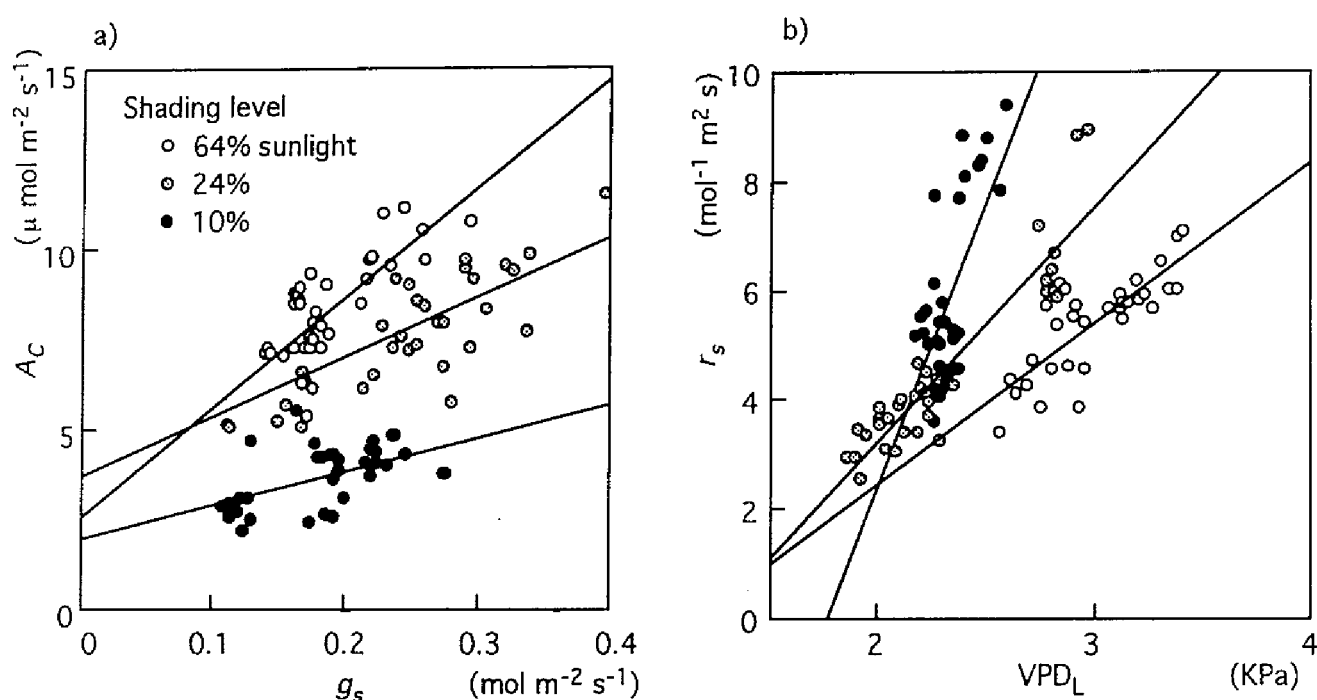


Fig. 3-2-7. Diurnal changes in photosynthetic photon flux density (PPFD), leaf CO<sub>2</sub> assimilation rate ( $A_C$ ), stomatal conductance ( $g_s$ ), relative humidity (RH), transpiration rate ( $E$ ), leaf to air vapor pressure deficit ( $VPD_L$ ), water use efficiency (WUE), and leaf temperature ( $T_L$ ) of cherimoya leaves grown under 64%, 24% and 10% sunlight environment. Measurements were made on July 13 1996.

among the treatments were not as large as those in  $A_C$ . Thus, WUE at 10% sunlight during daytime was substantially lower than less shading levels. At 64% sunlight condition,  $T_L$  for daytime was continuously higher. Time course changes in RH of the three shadings were very similar and indicated a reduction trend to the evening varying from 80% to 50%. It began to increase again after sunset. Increasing trends of  $VPD_L$  were observed until 1400 h, under all the conditions, and thereafter  $VPD_L$  decreased. At 64% sunlight, the statistically highest  $VPD_L$  was continued for 0800 h - 1400 h. There was non-significant differences between 24% and 10% sunlight.



**Fig. 3-2-8.** Relationships between a) leaf  $CO_2$  assimilation rate ( $A_C$ ) and stomatal conductance ( $g_s$ ), and b) stomatal resistance ( $r_s$ , inverse of  $g_s$ ) and leaf to air vapor pressure deficit ( $VPD_L$ ) of cherimoya leaves grown under different shading (64%, 24%, and 10% sunlight) conditions during the midday hours (1000 h - 1400 h). Solid lines are linear regression curves. The equations and correlation coefficients ( $r$ ) of the regression curves are described as follows: a) 64%:  $A_C = 30.349 g_s + 2.556$ ,  $r = 0.771^{**}$ ; 24%:  $A_C = 16.395 g_s + 3.739$ ,  $r = 0.724^{**}$ ; 10%:  $A_C = 9.2845 g_s + 2.011$ ,  $r = 0.514^{**}$ ; b) 64%:  $r_s = 2.946 VPD_L - 3.437$ ,  $r = 0.797^{**}$ ; 24%:  $r_s = 4.298 VPD_L - 5.337$ ,  $r = 0.925$ ; 10%:  $r_s = 10.45 VPD_L - 18.48$ ,  $r = 0.694^{**}$ .

During midday hours,  $A_C$  was linearly correlated to  $g_s$  (Fig. 3-2-8a). As light intensity increased, decreasing  $g_s$  had more direct effect on limiting  $A_C$ . There was close linear

correlation between  $r_s$  and  $VPD_L$  (Fig. 3-2-8b). The increasing  $VPD_L$  resulted in increasing  $r_s$ . This tendency was observed for all the conditions. The increasing rate of  $r_s$  caused by increasing  $VPD_L$  was higher as shading level increase.

### Leaf water potential

The leaves less exposed to the sun maintained higher level of leaf water potential throughout the daytime (Fig. 3-2-9). Leaves under each condition showed reducing leaf water potential to 1400 h, with some fluctuation. The water potentials reached a minimum at around 1400 h, and then it began to increase toward the night.

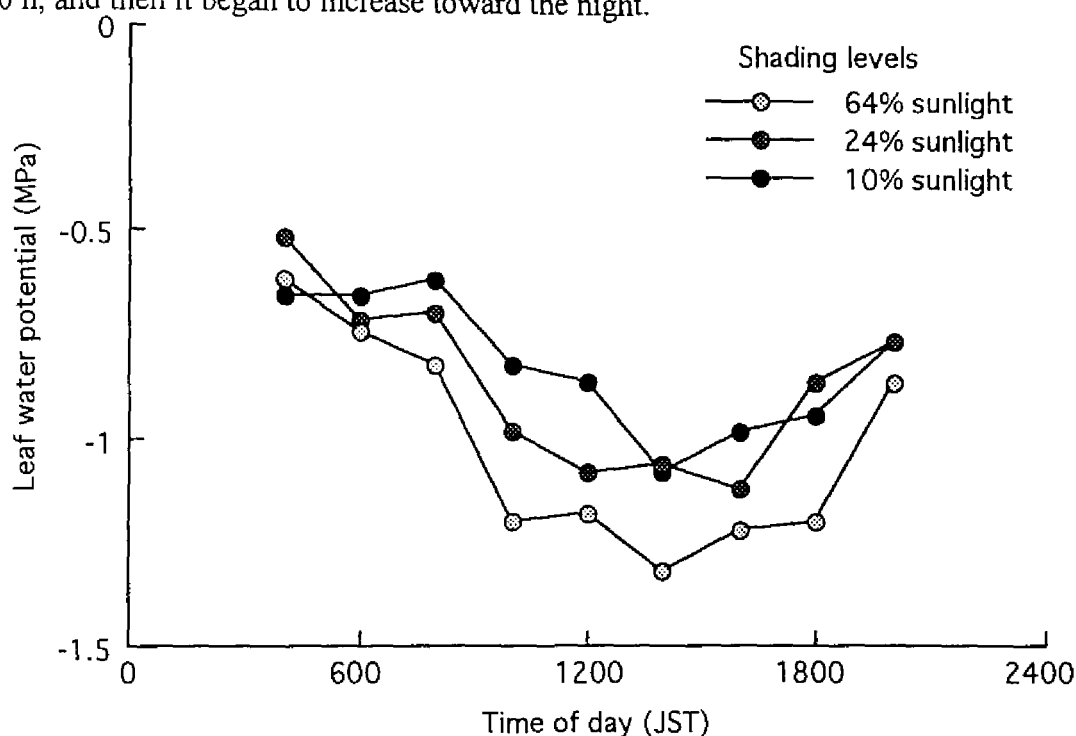


Fig. 3-2-9. Diurnal changes in leaf water potential as measured by a dew point psychrometer. Leaves for the measurement were sampled from cherimoya trees grown under different shading (64%, 24%, and 10% sunlight) conditions.

## DISCUSSION

Plants are capable of adaptation to their light environmental conditions (Berry, 1975). But the range of the adaptability is varied for each plant. As is noted in the Chapter 2, cherimoya seedling was the most shade-tolerant among three commercially important *Annona* trees:

sugar apple, soursop, and cherimoya. In the present experiment, fruit bearing cherimoya trees exhibited similar shading responses as the seedlings examined in Chapter 2, although a little reduction of shade tolerance was observed in mature trees.

Shading decreased shoot length, leaf number, stem diameter, and increased area per leaf. As shading increased, leaves became thinner and larger. Shading produced higher specific leaf area. Stems grown in the lower light were shorter and slenderer, but had the longer inter-node length, and resulted in larger specific stem length. Such morphological responses were similar to other tropical fruit trees such as carambola (Marler *et al.*, 1994) and mangosteen (Wiebel *et al.*, 1994). Deep-shaded cherimoya trees had a more horizontal leaf orientation. These modifications of thinner and larger leaves with more horizontal orientation are common adaptation to low irradiance (Kozlowski and Pallardy, 1997; Fitter and Hay, 1981). This adaptation is a plant strategy to maximize light interception, and the greater specific stem length and specific leaf area could explain it.

Leaf chlorophyll content on the basis of unit area was increased with reduced light level. This is also assumed to be an acclimation to low light environment to enhance the light use efficiency. Shade-tolerant plants are known to increase chlorophyll in leaves at low irradiance, whereas in shade-intolerant plants the chlorophyll content on a unit leaf area basis decreases or remains constant (Thompson *et al.*, 1992; Marler *et al.*, 1994). There were no significant differences in leaf chlorophyll content on the basis of unit area for mangosteen grown in 20% to 80% sunlight levels (Wiebel *et al.*, 1994). However, chlorophyll content for mango leaves on area basis increased as shade increased (Schaffer and Gaye, 1989a). Citrus leaves grown under heavy shade decreased maximum assimilation rate (Syvertsen, 1984) and decreased the thickness but increased chlorophyll content (Syvertsen and Smith Jr., 1984a). Cherimoya post-shade leaf grown at heavy shade (10% full sun) exhibited lower area-basis chlorophyll content than less shaded conditions, although pre-shade leaf in 10% sunlight indicated the highest chlorophyll content. This suggests that the shading level of 10% sunlight is beyond the adaptable range for cherimoya, although cherimoya can survive over a wide range of irradiance. Cherimoya is probably more shade-tolerant than mango but less than mangosteen.

High light intensity caused high  $T_L$  (Fig. 3-2-5), which was assumed to be a main factor to increase  $VPD_L$ . Increasing  $VPD_L$  tends to decrease  $g_s$  and  $A_C$  (Khairi and Hall, 1976; Farquhar and Sharkey, 1982; Higgins *et al.*, 1992). In the present experiment, cherimoya leaves decreased  $g_s$  and lowered  $A_C$ , during midday, with high  $VPD_L$  (Fig. 3-2-5). Similarly in Fig. 3-2-8, high  $VPD_L$  caused low  $g_s$  and then high  $r_s$  (inverse of  $g_s$ ) caused low  $A_C$ . The  $VPD_L$  of cherimoya is suggested to rise easily and to lead to stomatal closure under high irradiance. Accordingly, efficient light use under high light conditions might be difficult and  $A_C$  was limited for cherimoya.

Diurnal decline in  $g_s$  throughout daytime might be associated with increasing  $VPD_L$  (Fig. 3-2-7). George *et al.* (1990) noted that the midday suppression in  $g_s$  for Atemoya recovered with increasing relative humidity after 1600 h. Decreased  $g_s$  during midday even for non-irrigated macadamia leaf began to increase at 1600 h. Whereas for a cherimoya leaf, diurnal decline in  $g_s$  under various shading levels was consistently observed after sunset. On the contrary, citrus leaf tended to maintain high  $g_s$  under strong daylight (Moreshet, 1984). The diurnal  $g_s$  response of cherimoya was similar to that of coffee (Gutierrez *et al.*, 1994) and mangosteen (Wiebel *et al.*, 1994). Fanjul *et al.* (1985) also reported that  $g_s$  of coffee was continued to be restrained over afternoon on the day when  $VPD_L$  increased to 0.8 KPa. The stomata of cherimoya is suggested to be more prone to close than to open when exposed to midday atmospheric drought condition as compared to Atemoya, macadamia, and citrus. However, it is assumed to be less sensitive than litchi. The heavy suppression of  $g_s$  for litchi under high irradiance rapidly recovered in the evening (Menzel and Simpson, 1986b).

Water stress was closely related to midday acceleration of  $VPD_L$  with  $A_C$  suppression for citrus (Brakke and Allen, 1995). It is well known that water stress causes stomatal closure. The decline of leaf water potential during daytime in cherimoya indicated leaf water stress. The daytime reduction of leaf water potential was more pronounced under less shaded conditions. The lowered  $A_C$  during midday might be thus affected by stomatal closure with water stress. Decreased  $g_s$  in this situation is supposed to be a water conserving mechanism at the expense of  $A_C$ . This mechanism is assumed to interfere with light use efficiency of 64%

sunlight during midday. This assumption can be explained by the little difference in daytime WUE under 64% and 24% sunlight treatments.

The shading of 10% sunlight was beyond acclimation region for cherimoya. Low daily  $A_C$  in 10% sunlight was directly resulted from light limitation. In cherimoya, WUE in 10% sunlight was far lower than that in 64% or 24% sunlight. The effect of light deficiency on  $A_C$  was greater than the effect of  $g_s$  reduction, although decreasing trend in  $g_s$  under heavy shade was also reported in well-irrigated avocado (Sterne *et al.*, 1977). The same tendency in reduction of WUE was observed for mango in 24% sunlight (Schaffer and Gaye, 1989b), confirming the assumption that cherimoya leaf is more shade-tolerant than mango.

There was little difference in daily  $A_C$  between 100% and 47% sunlight for carambola (Marler *et al.*, 1994). Although substantial photoinhibition in 100% sunlight caused midday suppression in  $A_C$  for carambola, tree in 47% sunlight indicated no reduction in midday  $A_C$ . The reduction by low irradiance was observed during early morning and late afternoon hours. In cherimoya, midday  $A_C$  was similar in 24% and 64% sunlight, although  $A_C$  in 100% sunlight was much lower than in 24% and 64% conditions (Fig. 3-2-5), indicating heat damage against  $A_C$  at full sunlight. However, daily total  $A_C$  in 64% sunlight was apparently higher than in 24% (Fig. 3-2-7). In the morning, high  $g_s$  enabled to maximize quantum yield and thus  $A_C$  was higher under less shaded condition. During late afternoon hours, light intensity in 24% sunlight was insufficient for  $A_C$ .

The reduction in  $A_C$  in the present results may not be caused by photosynthetic reaction center damage, but caused by stomatal closure, although further experiments concerning chlorophyll fluorescence or active oxygen measurement are needed to confirm this. Gamon and Pearcy (1990) noted that photoinhibition in PSII reaction center for *Vitis californica* was began to be observed at high leaf temperature above 45°C and at high light above 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Increase of active oxygen may damage photosynthetic membranes under high temperature with high light (Asada and Takahashi, 1987). However, the situation of actual cultivation with such a high leaf temperature is unusual for commercial orchard.

Shading may be effective for decreasing leaf temperature, easing water stress and

prevention the danger of photoinhibition. Shade leaves typically increase quantum use efficiency through physiological adaptations (Fitter and Hay, 1981). Adequate levels of shading were effective to maintain relatively lower leaf temperature and higher  $g_s$ . The effect of shading varied from 24% to 64% sunlight on increasing  $g_s$  may compensate for the effect of low light intensity, attributing to increase  $A_C$ .

## SUMMARY

Six-year-old cherimoya trees were grown under plastic house at 3 different shade conditions (64%, 24%, and 10% of full sunlight). Shoot length, shoot diameter, leaf number, and total leaf area were decreased by shading, but inter-node length and single-leaf area were increased. Shading produced larger and thinner leaves. Leaf chlorophyll content was increased at 24% sunlight for both pre- and post-shade leaves. As compared with leaves under full sunlight, leaves at 64% and 24% sunlight performed higher  $A_C$  with higher  $g_s$ . Under full sunlight, high leaf temperature caused high leaf vapor pressure deficit, resulting in reduction of gas exchange rate. The higher  $A_C$  was achieved at 64% and 24% sunlight. Throughout the daytime,  $A_C$  at 64% sunlight was maintained at the highest level except for during midday, when  $g_s$  and leaf water potential at 64% was significantly decreased. Air temperature and relative humidity were little affected by shading.



## Reproductive growth as affected by high temperature

### Section 1.

## Floral differentiation and flowering

### INTRODUCTION

As described in the previous chapters, cherimoya is supposed to be difficult to cultivate in the tropics. A trial introduction into Florida resulted in no success with any variety or seedling (Popenoe, 1974). In the Okinawa islands, located in sub-tropical Japan, the fruit production has also not been successful due to poor flowering, despite two flowering periods a year.

Investigation as to the effects of temperature on floral bud induction and on subsequent flowering is essential for better understanding the high temperature inhibition on fruit productivity. Few studies related to cherimoya floral bud formation as affected by temperature can be found, although some studies concerned with *Annona* pollination at developmental temperatures are available (e.g. Utsunomiya *et al.*, 1992a; George and Nissen 1988; Thakur and Singh, 1965; Schroeder, 1943).

Floral initiation requires low temperatures for many fruit trees. Winter chilling temperatures are often thought to be the main trigger for floral initiation in temperate fruit trees such as apple (Tromp, 1980) and citrus (Moss, 1969). For some tropical and sub-tropical fruit trees, high temperature suppression of flowering has also been reported for litchi (Menzel and Simpson, 1995), mango (Whiley *et al.*, 1989) and avocado (Sedgley *et al.*, 1985). These trees, however, varied widely in their flowering responses to temperature. Chaikiattiyos *et al.*

(1994) noted that mango trees were induced no flowers above 15°C, while in avocado the optimum temperature for flowering was 25°C (Sedgley *et al.*, 1985). For cherimoya, no previous study has been conducted to investigate flowering responses under tropical and subtropical temperature conditions.

Marler and Crane (1994) conducted a study related to the primary bud complex of atemoya, a hybrid between cherimoya and sugar apple, but they did not mention the influence of ambient temperature. The specification of the floral initiation period is important for evaluating the temperature effect on flowering, since the temperature during floral differentiation appears to affect floral number and size (Batten and McConchie, 1995; Susanto *et al.*, 1991).

The anthesis of deciduous trees usually occurs more than 7 months after floral initiation, while that of ever-green trees does within 3-months (Inoue and Takahashi, 1989). Cherimoya is a semi-deciduous tree (Morton, 1987), hence the pattern of floral bud formation is presumed to have both deciduous and ever-green characteristics. However, information on bud formation is very limited.

The objectives of this Section are to elucidate the floral differentiation pattern of cherimoya and to determine its flowering response to tropical and sub-tropical temperatures.

## **MATERIALS AND METHODS**

### ***Plant materials and treatments***

Three-year-old grafted 'Big Sister' cherimoya trees were used. The trees, planted in 20 l plastic pots filled with a mixture of sand and loam (1:1, v/v), were grown in a plastic-house where the temperature was maintained above 12°C. In December 1994, these plants were transferred to two bays of a naturally lit glass-house where the day/night temperatures were maintained at 20/15°C and 30/25°C. The duration of day temperatures was 12 h, and plants received daylight which varies seasonally from 9.8 h in winter to 14.5 h in summer. Relative humidity varied from 50% to 80% in both bays. Seven plants were used for each treatment. Each plant was irrigated daily.

The experiment continued for two years. On March 1 1995, every tree was pruned with only 3 stems left cut back to 5 basal nodes. All leaves were then artificially defoliated. On the same day in the next year, the trees were pruned again leaving 6 stems per tree, followed by the defoliation in the same manner as in the previous year.

### ***Floral bud differentiation in leaf axil***

In both treatments, axillary buds for microscopic examination were obtained at different leaf ages (1, 2, 4, 8 weeks after unfolding, and just unfolding), on July 5 1996. A series of continuous cross sections was made by a microslicer (DTK-1000, D.S.K.) at a thickness of 50  $\mu\text{m}$  without fixation or staining. The section facet was vertical and included the petiole and stem surrounding the axillary bud.

The numbers of floral and vegetative buds, and undifferentiated primordia per axillary bud complex were counted. In each bud complex, the diameter and height of the largest floral and vegetative buds in the central-cross section were measured using objective and eyepiece micrometers (Fig. 4-1-1).

### ***Flowering number, period, and nodal position***

The number of flowers, flowering date, days to visible emergence of floral buds, and the nodal positions of flowering were recorded for all the flowers at both temperature regimes for two years. Flowering at the male stage (Vithanage, 1984) was used to determine the flowering date, number and position. Nodal positions were numbered consecutively, from the basal to the upper most position of shoots. The time course changes of flowering number for every 5-day period from April 1 were determined. At the same time, the number of flowers at each nodal position was counted. If flower buds dropped before the anthesis, only the numbers and nodal positions were recorded. On June 1 1995, 10 shoots were randomly selected from each temperature regime and the number of nodes per shoot were counted.

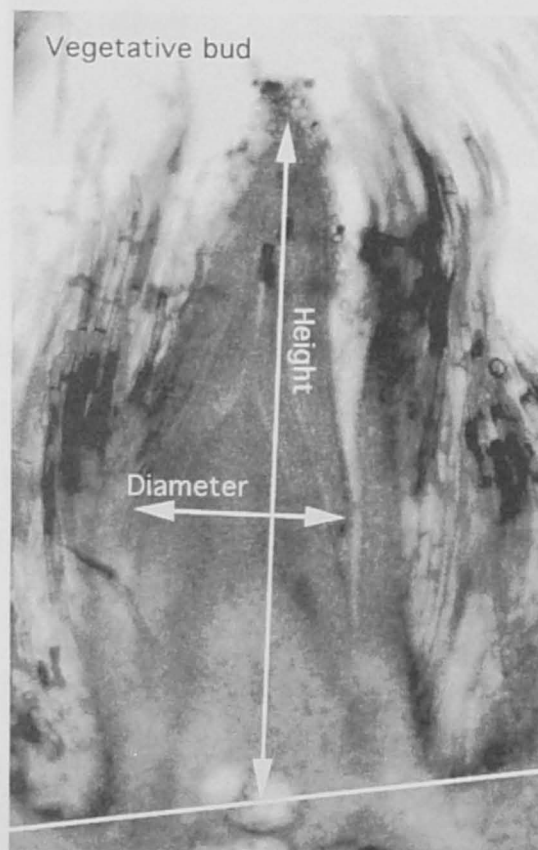
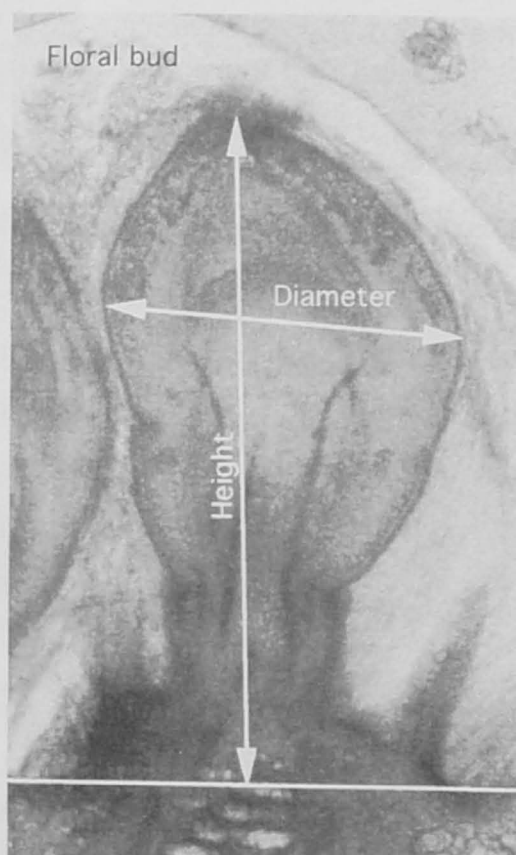
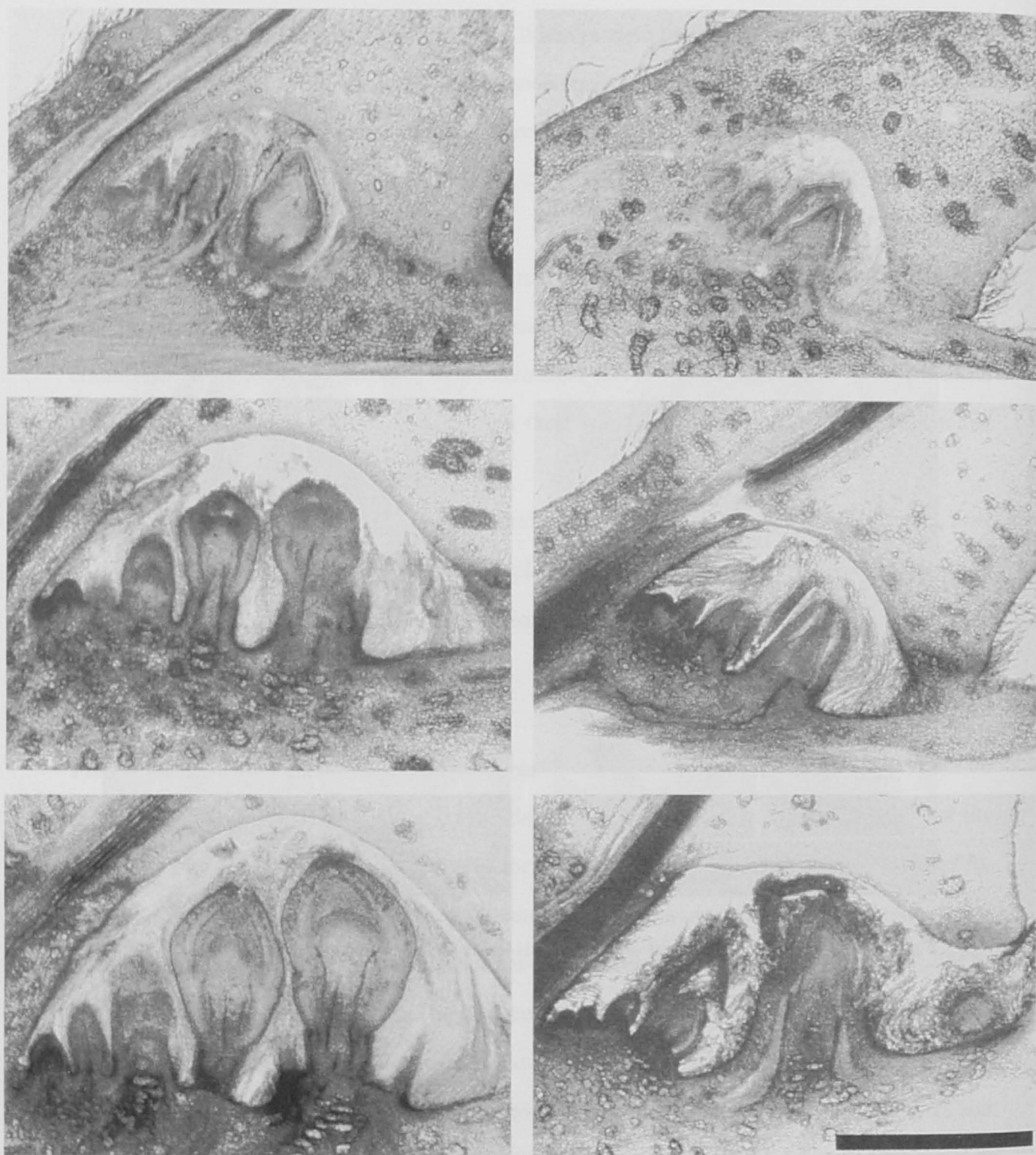


Fig. 4-1-1. Dimension of floral and vegetative buds inside axillary bud complex.



**Fig. 4-1-2.** Vertical section of cherimoya axillary bud complex at developmental stages behind leafstalk at 1, 2, and 4 (top to bottom) weeks after unfolding under 20/15°C (left) and 30/25°C (right) day/night temperature regimes. The scale bar represents 1 mm.

### ***Floral morphology***

In April 1995 and 1996, 10 male stage flowers at the basal nodes were randomly picked from both temperature regimes. After measuring the fresh weight of each flower (excluding the peduncle), the three major petals and compounded pistil were separated. The middle value length and width of the petals, total fresh weight of the three petals, and fresh weight and diameter of the compounded pistil were then measured. After the measurements, the ratio of pistil to the whole flower weight was calculated.

## **RESULTS**

### ***Floral bud differentiation in leaf axil***

The axillary bud complex at developmental stages is shown in Fig. 4-1-2. In the axil of the just unfolded-leaf stage, neither floral nor vegetative buds differentiated. A floral morphogenesis was observed inside one-week-old leafstalks, although some primordia at the axil were found to be indistinguishable stage between floral and vegetative buds. Apical meristem did not produce floral bud at 30/25°C (Fig. 4-1-3).



Fig. 4-1-3. Distal flower bud formation around apical meristem observed at 20/15°C (left), but not at 30/25°C (right).

The total numbers of differentiated buds per leaf axil increased until 4 weeks after unfolding at both temperature regimes, and then remained constant thereafter (Fig. 4-1-4). More floral buds per axil developed at 20/15°C than at 30/25°C. At 20/15°C, although the number of floral buds was more than vegetative buds at any stage, both the floral and vegetative buds increased at the same rate. At 30/25°C, however, the floral bud number remained at the markedly lower level. On the other hand, the vegetative bud number greatly increased at 30/25°C, where most buds developed to be vegetative. At the axil of 8-week-old leaf, only 0.5 average floral buds were produced at 30/25°C, while there were 2.4 floral buds observed at 20/15°C. At both temperature regimes, although the percentage of indistinguishable young buds decreased through time, the young buds were continuously observed to be 20-25% of total buds.

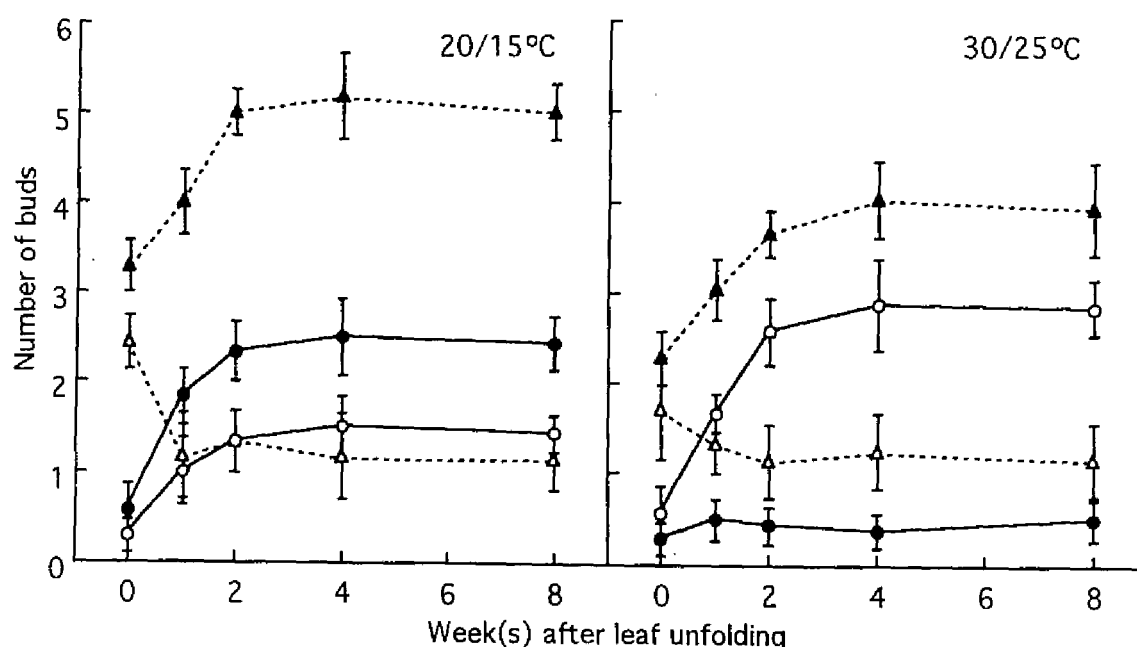


Fig. 4-1-4. Number of floral and foliar buds developed per axillary bud complex 0 to 8 weeks after unfolding.  
 —●— Floral bud —○— Vegetative bud ---△--- Undifferentiated bud ---▲--- Total

**Table 4-1-1.** Plumule dimensions of floral and vegetative buds in a bud complex of the leaf axilla aged 0 to 8 weeks after leaf unfolding.  
Data include the dimensions of young buds which had not developed enough to be identified as floral or vegetative.

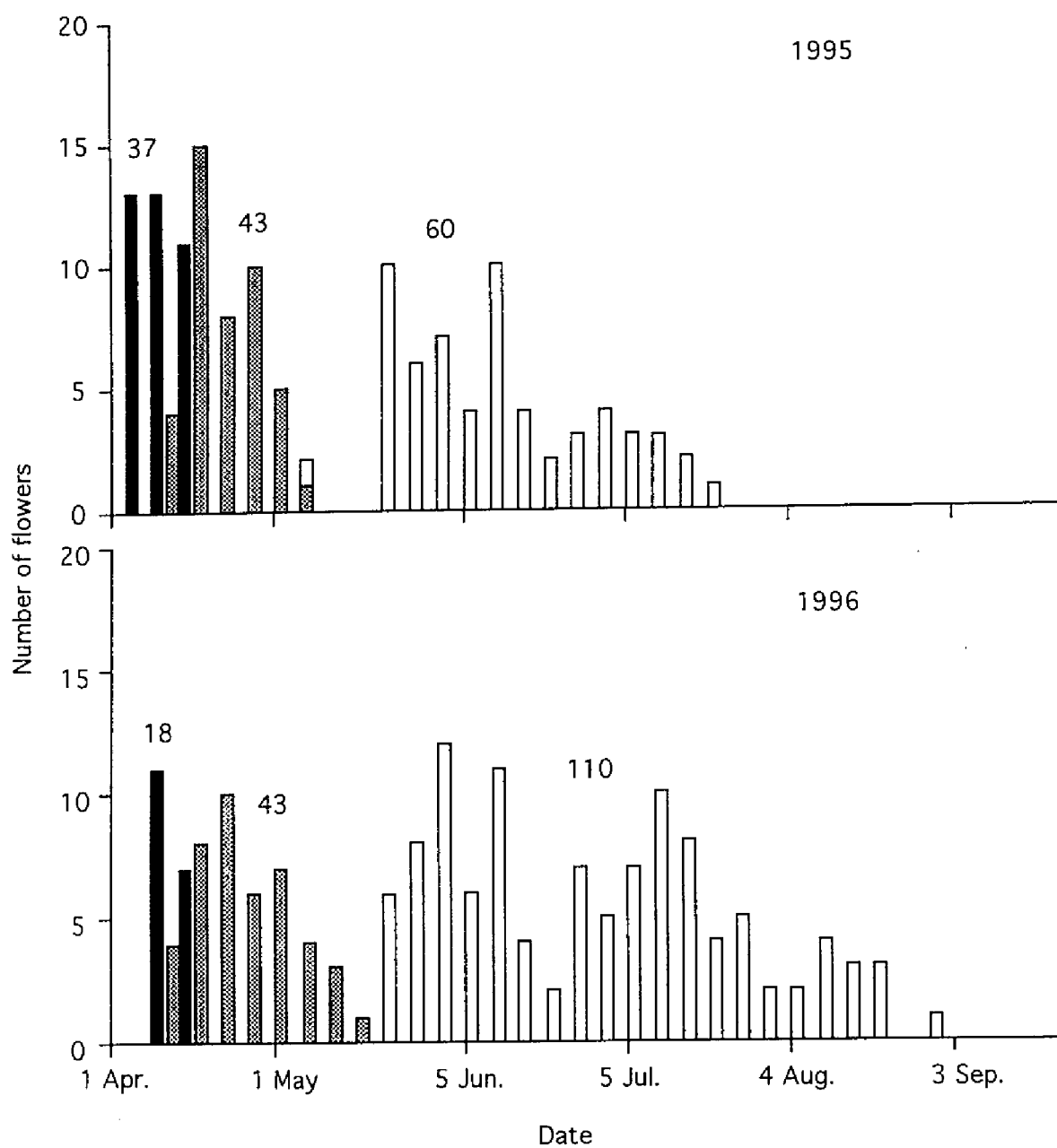
Variables		Day/night temperatures (°C)	Week(s) after leaf unfolding				
			0 <sup>2</sup>	1	2	4	8
Diameter (μm)	Floral bud <sup>y</sup>	20/15	342 ± 45	526 ± 75	757 ± 109	863 ± 105	989 ± 79
		30/25	279 ± 41	395 ± 25	381 ± 74	642 ± 54	594 ± 81
	Vegetative bud	20/15	342 ± 45	239 ± 17	301 ± 47	250 ± 26	274 ± 28
		30/25	279 ± 41	338 ± 12	226 ± 21	262 ± 22	306 ± 34
Height (μm)	Floral bud	20/15	442 ± 78	944 ± 130	1308 ± 181	1463 ± 173	1724 ± 176
		30/25	363 ± 49	715 ± 35	819 ± 132	1183 ± 194	1018 ± 92
	Vegetative bud	20/15	442 ± 78	468 ± 27	566 ± 116	540 ± 66	709 ± 138
		30/25	363 ± 49	588 ± 112	576 ± 16	617 ± 83	686 ± 129

<sup>2</sup> A bud complex inside the axilla of leaves just unfolding.

<sup>y</sup> Maximum diameter of the largest floral bud inside the single bud complex.

Data are mean ± SE.





**Fig. 4-1-5.** Every five-day-total number of flowers in cherimoya at 30/25 °C and 20/15°C day/night temperature regimes for 2 continuous years. Numerals above graph bars indicate the number of flowers.

□	20/15°C	Flowers at the 4th or more distal node.
▨	20/15°C	Flowers at the 1st to 3rd nodal positions.
■	30/25°C	Flowers at the 1st to 3rd nodal positions.

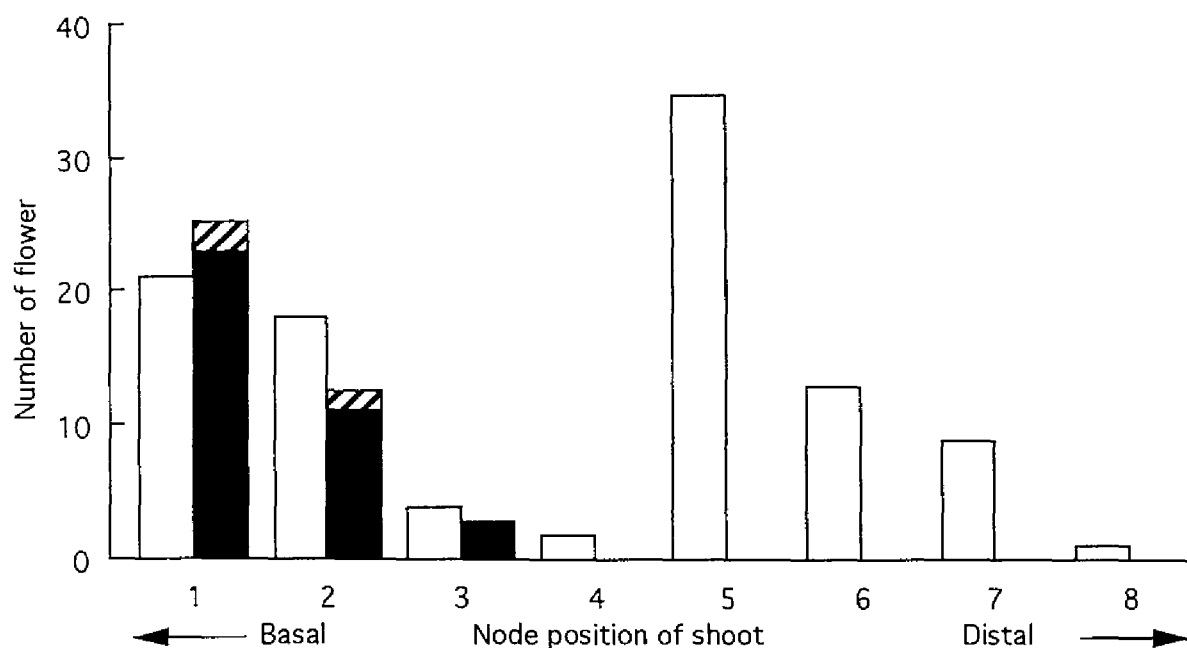


Fig. 4-1-6. Number of flower buds produced at each nodal position of cherimoya shoots under 30/25 °C and 20/15°C day/night temperature regimes in 1995.

□ 20/15°C    ■ 30/25°C    ▨ Dropped buds before anthesis at 30/25 °C

Floral buds at 20/15°C grew larger in size than at 30/25°C (Table 4-1-1). On the other hand, there was little difference in vegetative bud size in the two different temperature regimes.

### ***Flowering number, period, and nodal position***

The number of flowers was much greater at 20/15°C than at 30/25°C (Fig. 4-1-5). The limited number of flowers at high temperatures was more apparent in the second year than in the first year. Flowers at 20/15°C increased in the second year, while flowers at 30/25°C decreased more appreciably than in the first year. The flower numbers in the first and second year were 103 and 153 at 20/15°C, and 37 and 18 at 30/25°C, respectively.

In both years, the flowering period at 30/25°C started about a week earlier than at 20/15°C, and the duration was very shorter at 30/25°C. In the first year, the flowering at 20/15°C and 30/25°C started at 42 and 33 days after defoliation, respectively, and the duration was 104 at 20/15°C and 13 days at 30/25°C (Table 4-1-2). In the second year, the beginning of the

flowering season at both temperatures was very similar to the first year, but the duration of flowering at 20/15°C extended to 158 days while at 30/25°C, the flowering period was reduced to 10 days. The trees at 30/25°C regenerated a second flushing in October of the second year, however only a few flowers were produced at the basal nodes of new shoots.

**Table 4-1-2.** Flowering and bud development period as affected by 20/15°C and 30/25°C day/night temperatures through two years.

Variables	1995		1996	
	20/15°C	30/25°C	20/15°C	30/25°C
Start of flowering season	Apr.12	Apr.3	Apr.14	Apr.6, (Oct.24)*
End of flowering season	Jul.24	Apr.15	Sep.18	Apr.15, (Oct.31)*
Duration of flowering season (days)	104	13	158	10+(8)*
<b>Flowers on the basal to the 3rd nodes</b>				
Days after differentiation to emergence	11 months	11 months	11 months	11 months
after emergence to flowering (days) <sup>2</sup>	42	33	44	36
Start of flowering season	Apr.12	Apr.3	Apr.14	Apr.6, Oct.24
End of flowering season	May.6	Apr.15	May.19	Apr.15, Oct.31
Duration of flowering season (days)	25	13	36	10, 8
<b>Flowers on the 4th to distal nodes</b>				
Days after pruning to differentiation (days)	33	nil**	40	nil
after differentiation to flowering (days)	35	nil	41	nil
Start of flowering season	May.8	nil	May.21	nil
End of flowering season	Jul.24	nil	Sep.18	nil
Duration of flowering season (days)	78	nil	121	nil

<sup>2</sup>Floral buds appeared at the leaf scar with artificial defoliation.

\*Flowering with the secondary shoot flushing.

\*\*No flower

The developmental period from defoliation to the visible emergence of distal buds (produced at the 4th or more distal node of the shoot) was 33 and 40 days, in the first and second year, followed by a period of 35 and 41 days from the emergence to the flowering. From the axillary bud complex at every scar, floral and vegetative buds emerged to be visibly recognizable within a week after defoliation.

At 20/15°C, flowers were formed from the 1st to the 8th nodes on the shoots (Fig. 4-1-6). The largest number of flowers at 20/15°C were produced at the 5th node. On the other hand, flower formation at 30/25°C was limited to the 1st to 3rd nodes. Among these flowers, the more flowers were observed at the more proximal node. The number of these basal flowers

differed little between temperature regimes. The differentiation of the distal floral buds occurred around the apical meristem. These buds came out one after another from each meristem of the bud complex. On July 1, the average node number was 8.5 at 20/15°C and 7.5 at 30/25°C per shoot.

### ***Floral morphology***

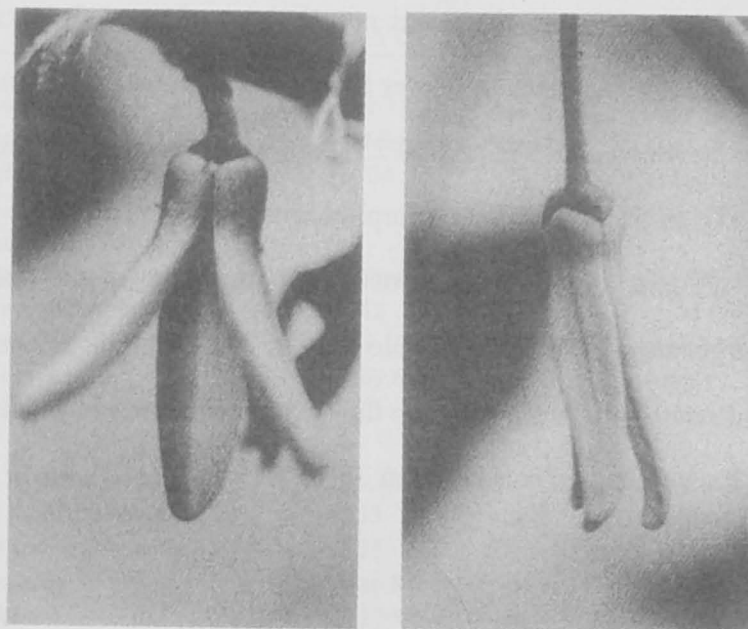
Temperature had less effect on the floral morphology of basal flowers in the first year. There were no significant differences in flower fresh weight, petal length and width, and pistil diameter and weight between 20/15°C and 30/25°C, however only petal weight was heavier at 20/15°C (Table 4-1-3). In the second year, morphological differences of the floral organs were larger (Fig. 4-1-7). Weights and dimensions of the whole flower, petal, and pistil at 20/15°C were statistically larger than at 30/25°C (Table 4-1-3). Pistil weight percentage in the whole flower was not significantly different between the two temperatures in the first year. However, in the second year, the percentage was larger at 30/25°C.

**Table 4-1-3.** Effect of 30/20°C and 20/15°C day/night temperature regimes on flower weight, petal and pistil size of cherimoya flowers at female stages.

Variables	1995		1996	
	20/15°C	30/20°C	20/15°C	30/20°C
Flower fresh weight (g)	1.44	1.29	1.93*	0.84
Petal length (mm)	31.8	33.5	39.8*	28.7
width (mm)	6.88	6.64	7.22*	4.56
fresh weight (g)	1.30*	1.16	1.70*	0.75
Pistil diameter of compound carpel (mm)	3.91	3.77	4.09*	3.34
fresh weight (mg)	13.7	12.6	15.9*	8.9
Pistil percentage per flower (% fw/fw)	9.49	9.74	8.25	10.6*

\*: significant difference within a year at  $p < 0.05$  level by t-test.

Data are means of 10 replications.



**Fig. 4-1-7.** Cherimoya cv. Big Sister flowers at 20/15°C (left) and 30/25°C (right) day/night temperatures observed in the second year of the temperature treatment.

## DISCUSSION

### *Floral bud formation patterns*

Two different morphogenesis processes of cherimoya resulted in flowering at different positions. Floral morphogenesis occurred both in the long-dormant axillary buds and in the terminal buds of the extending shoots. The axillary differentiated floral buds emerged at the basal nodes of the next season shoots and apically differentiated buds were located at the distal nodes of the flushing shoots.

A multiple bud complex is common in tropical trees (Hallé *et al.*, 1978). In this study, cherimoya tree was found to produce a multiple bud complex consisting of several floral and vegetative meristems developed separately in a leaf axil. A few floral buds, which had been initiated in a leaf axil, emerged with one or two flushing shoots and were located at the basal nodes on the shoots. This process of floral bud development was similar to that of deciduous trees. However, the cherimoya tree also formed an apically produced bud complex at distal part of the shoot. Such synchronization of floral differentiation with shoot flushing was similar to that of guava (Menzel and Paxton, 1986) and passionfruit (Menzel *et al.*, 1987), although the flowers of cherimoya were formed on the opposite side of the leaf.

### *Flower number*

Some articles cited in the previous chapters also shows that high temperatures over 30°C reduced or inhibited the floral induction of tropical and sub-tropical fruit trees such as litchi (Menzel and Simpson, 1995), mango (Whiley *et al.*, 1989), avocado (Sedgley *et al.*, 1985), and pummelo (Susanto *et al.*, 1991). Shu and Sheen (1987) found that the number of axillary buds of mango increased with decreasing day/night temperatures ranging from 31/25°C to 19/13°C. They observed no flowering at 31/25°C. In cherimoya, although flowers were largely reduced at 30/25°C, this reduction was mainly attributed to the floral inhibition at the shoot apex. At 30/25°C, no flowers were induced at distal nodes. However, the number of basal flowers was marginally affected by the similar temperature regimes to those observed by Shu

and Sheen (1987).

Susanto *et al.* (1991) pointed out that the temperature after floral differentiation had a marginal effect on the number of emerged flower buds for pummelo. A similar tendency was also observed for cherimoya. Temperature had little effect on the number of basal flowers in the first year. In the second year, however, high temperatures reduced the number of basal flowers. These flowers differentiated in the leaf axils about a year before flowering, as indicated by the microscopic examination. These buds were waiting in a dormant stage behind leafstalks for the following defoliation. It was, thus, before the start of temperature treatments that the basal flowers in the first year had differentiated. Thus, high temperatures had little effect on the number of basal flowers in the first year, but decreased the number in the second year. The larger number of basal flowers at low temperatures in the second year was supported by the larger number of floral buds differentiated in the axils in the first year (Fig. 4-1-2).

### ***Floral morphology***

Flowers tend to grow larger in cool environments for lychee (Menzel and Simpson, 1995) and for some tropical fruit trees (Chaikiattiyos *et al.*, 1994). George *et al.* (1994) explained that the larger flowers of persimmon grown at low temperatures resulted from the long developmental period. Poerwanto and Inoue (1990) also reported that floral organs of satsuma mandarin developed under cool conditions were larger due to a slow growth rate. In this study, flowers at low temperatures became significantly larger and the developmental period was longer than at high temperatures in the second year. However, the longer developmental period in the first year under cool environments did not produce larger flowers. This was because the temperature sensitive periods for floral size were before the start of the temperature treatments. Basal flower size was predetermined during the axillary bud stage. The axillary floral buds at high temperatures were significantly smaller than at low temperatures nearly a year before the anthesis (Table 4-2-1). The temperature sensitive period for floral size was suggested to be during the floral differentiation as consistent with the sensitive period for floral number mentioned above.

### ***Flowering period***

It has been reported that flowering periods for satsuma mandarin (Inoue, 1989) and persimmon (George *et al.*, 1994) were shortened at high temperatures, as observed in this experiment. In cherimoya, high temperatures markedly reduced flowering periods, mainly because the flower buds were formed only on the basal nodes. Whereas at low temperatures, however, the distal flowering continued after the completion of basal flowering in succession with the growth of flushing shoots.

Two flowering periods in the second year were observed in April and August at high temperatures, although the total time frame was still significantly short, consistent with the situation observed in the Okinawa islands. A similar flowering behavior has been reported in India (Venkataratnam, 1959).

High temperatures resulted in an early and short flowering season for litchi (Menzel and Simpson, 1991) and avocado (Sedgley *et al.*, 1985). These flowers grew faster in warmer environments. Short term flowering period at high temperatures can be reflective of fast developing rate of floral organs. Similarly in cherimoya, the reduced anthesis duration and early flowering were also indicative of fast floral development. The flowerings at 30/25°C continued for 13 and 10 days in the first and second year, respectively. Whereas, flowering period at 20/15°C was 25 days for the first and 36 days for the second year, if the flowering is limited to 1st to 3rd nodes as at 30/25°C. This indicates that the flowering period at high temperatures was distinctly shorter, even when compared on the basis of the same nodal position with the similar number of flowers.

Generally, tropical or sub-tropical fruit trees commence flowering after a relatively briefer developing period. The duration from the differentiation to the anthesis for tropical and sub-tropical trees such as macadamia was 5 months (Moncur *et al.*, 1985), kiwi fruit 2 months (Watanabe and Takahashi, 1984), passionfruit 2 months (Ishihata *et al.*, 1989), avocado 1 - 2 months (Schroeder, 1951) and mango 1 month (Scholefield *et al.*, 1986). On the other hand, the durations for the temperate deciduous fruit trees were longer than those for tropical and



sub-tropical trees, at 9 months for persimmon (Harada, 1984), 8 months for apple (Li *et al.*, 1995) and pear (Banno *et al.*, 1986). Cherimoya tree, which is sub-tropical and also semi-deciduous, is rather unique in possessing both temperate deciduous and tropical evergreen bud developing patterns. Days to flowering of distal flowers after the differentiation were 35 and 41 days in the first and second year, and about 11 months for basal flowers. The distal buds had no dormancy, although the basal buds had a long dormant period during which the emergence was held on the axil until defoliation.

### ***Temperature for bud differentiation***

A certain level and term of low temperatures has been reported to promote floral differentiation and development for tropical or sub-tropical fruit trees such as citrus (Hall *et al.*, 1977), lychee (Menzel and Simpson, 1995), and avocado (Sedgley *et al.*, 1985). In cherimoya, the low temperature requirement for floral differentiation varied with the processes of bud morphogenesis. Distal nodes had no floral induction at high temperatures, suggesting that the optimal temperature for floral differentiation on these nodal positions was much lower than 30/25°C. This appears to be similar to other tropical or sub-tropical fruit trees. Satsuma mandarin trees grown at 25°C and 30°C produced vigorous vegetative flushing and no flower buds (Inoue, 1989). Temperatures below 25°C for avocado, and below 20°C for mango and litchi were reported to be essential for flowering (Chaikiattiyos *et al.*, 1994). On the other hand, basal flowers of cherimoya were induced even at 30/25°C, although the number was small. Floral induction even at 30/25°C through two years might lead to an expectation of every year flowering.

With a rather unusual characteristic of sub-tropical fruit trees, cherimoya can possibly provide flowering every year under tropical conditions. However, the cultivation under tropical condition is still not recommended. Short term flowering with few small flowers may cause unsuccessful fruit production. Although the fruit production potential of the distal flower was assumed to be less than the basal flower (George and Nissen, 1988), large number of distal flowers can contribute to a sufficient pollen supply. Moreover, well developed basal

flowers in cool environments will lead to a better fruit set.

## **SUMMARY**

To clarify the floral inhibition in cherimoya in warm environments, the number of flowers, floral morphology, nodal position, days to flowering, and flowering period were determined under 20/15°C and 30/25°C day/night temperature conditions throughout two years. Results showed that floral differentiation for cherimoya was more favorable at 20/15°C than at 30/25°C. More flowers were produced at 20/15°C and the flowering period was longer. Flowers were also larger at 20/15°C, however, the growth rate of floral organs was higher at 30/25°C. The temperature effects were more pronounced in the second year. The floral responses were different depending on the nodal positions, since different flower in its nodal position exhibited different developmental process. The floral buds at the basal point of shoot (at the 1st - 3rd nodes) differentiated almost a year previous to their anthesis and the distal buds (at the 4th - more distal nodes) differentiated, as synchronization with the shoot extension, about 5 weeks before anthesis. The floral buds for basal flowers of the next season had already differentiated even in one-week-old leaf axils. Cherimoya produced an axillary multiple bud complex for the upcoming blooming and flushing. In a 4-week-old leaf axil, a few floral buds with one or two vegetative buds usually developed at 20/15°C, while none or one floral bud with several vegetative buds developed at 30/25°C. The bud differentiation period was found to be the most temperature sensitive stage to influence flower number and morphology. There were no distal flowers induced at 30/25°C; and this was the main limitation on the number and on the period of flowering under tropical conditions.

## Reproductive growth as affected by high temperature

### Section 2.

## Fruit set and growth

### INTRODUCTION

The matter production and leaf assimilation, which are closely related to fruit production, were not increased for cherimoya at warm environment as seen in Chapter 2 and 3. The former Section found that continuous cultivation at high temperature resulted in reduction in the number of flowers and floral size. As for floral induction, warm environment of 30/25°C day/night temperatures was concluded to be disadvantageous.

In Japan, cherimoya cultivation is limited to warm climate areas where green-houses are used for protection from frost damage. In summer, however, green-house temperatures often exceed 30°C. Under such conditions, fruit yield and quality have been observed to be lowered (Yonemoto, 1996). Schroeder (1945) noted that the warm and dry environment in the interior valleys of California adversely affected pollination and fruit quality. Popenoe (1974) reported that cherimoya trees which had been brought into Florida grew well, but only produced a few misshapen “nubbins”.

Reliable fruit production by hand-pollination can be achieved using a large quantity of viable pollen (Richardson and Anderson, 1996). High temperatures, however, are thought to cause pollen sterility (Sweet, 1985). Information concerning cherimoya fruit set or fruit growth under high temperatures is very limited, although George and Nissen (1988) evaluated

temperature effects on the flowering and fruit set of atemoya, a hybrid of *Annona cherimola* X *A. squamosa*.

In this paper, pollen germination ability, fruit set, and subsequent fruit growth under high and low day/night temperatures were compared to clarify the mechanism of heat suppression of cherimoya fruit production and to obtain information on cultivation techniques that might improve production and reduce damage in warm environments.

## **MATERIALS AND METHODS**

### ***Plant materials and treatments***

Three-year-old grafted 'Big Sister' cherimoya trees were used for this experiment. These plants were grown in 20 l plastic pots containing a mixed medium of sand and loam (1:1, v/v). In December 1994, seven plants each were transferred to two bays of a naturally lit phytotron where day/night temperature regimes were maintained at 20/15°C (low) and 30/25°C (high). The day temperature duration was 12 h (from 0630 h to 1830 h), with the trees receiving daylight for 9.8 h to 14.5 h. Relative humidity varied from 50% to 80% in both bays. On March 1 1995, the trees were pruned and defoliated, leaving 3 stems with basal 5 nodes. In December 1995, further four trees of 4-year-old 'Big Sister' were set into each bay of the phytotron. These trees were pruned on March 10 1996, leaving 6 stems with basal 5 nodes, and all the leaves were removed. Each plant was irrigated daily and fertilized monthly.

### ***Pollen germination***

Pollen germination ability was examined from April 4 to 15 and from April 15 to May 28 in 1995, and from April 13 to 24 and from April 24 to May 6 in 1996, for high and low temperatures, respectively. Using a paint brush, pollen was collected from newly opened flowers at the male stage (Gazit *et al.*, 1982). Immediately after collection, the pollen was placed on a 2% agar medium containing 15% sucrose. Then, the placed pollen was kept in an incubator, where the temperature was maintained at either 20°C or 30°C. Consequently, there were four temperature treatments: two different pollen-development temperatures into two

different pollen-germination temperatures. The germination percentages were determined 24 h after the pollen placement by optical microscopic observation of 200 pollen grains. When the pollen tube grew longer than the pollen diameter, it was regarded as germinated. These examinations were repeated 5 times, by shifting the scope area.

### ***Fruit set***

To determine fruit set percentages, hand-pollination was conducted from April 5 to May 1 in 1995, during full bloom. There were four pollination treatments: using pollen developed at high or low temperatures into stigmas at high or low temperatures. The same treatments were also repeated in the next year, from April 7 to 25. Fresh pollen was collected from flowers at the male stage and pollinated flowers at the female stage which were considered ready for pollination (Gazit *et al.*, 1982). A small paint brush was used for collecting pollen and for hand-pollination (Richardson and Anderson, 1996). The brush was washed with ethyl-acetate every time after hand-pollination to prevent contamination. The numbers of flowers which dropped a week after pollination, and fruitlets which dropped within two months, were recorded. The number of irregular shaped fruits was counted on July 1.

### ***Fruit growth and development***

Fruit growth pattern was determined by measuring the horizontal diameter at two-week intervals from June 1 to the harvest, in 1995. The flowers were hand-pollinated to obtain fruit for this measurement, using pollen from the same regime as the flowers. The number of fruits measured throughout the season were 6 and 2, at low and high temperatures, respectively. The same measurements were repeated also in the following year, using fruits pollinated on April 15. Pollen from low temperatures, which displayed a germination percentage of 85% on the artificial medium described previously, was only used for fruit-set in the second year. Four single-fruit-bearing trees were growing at each regime. On July 1, two out of four trees in each bay were transferred to another regime. One fruit exhibiting the most typical growth pattern was selected from each treatment, both the years, to illustrate the growth curve. This singular

selection was used because characteristic growth patterns may be hidden by using averages (Abbott, 1984).

After measuring the fresh weight and diameter of the harvested fruits, their shapes were evaluated according to 5 levels of irregularity: 1 to 5, completely symmetric to completely irregular. The fruits were then left at room temperature for a week for softening. The total soluble solids content (TSS) of the softened fruits was measured by a refractometer (N1 , Atago Co. Ltd.), and the seed number in each fruit was counted. The seeds were then dried at room temperature for a week and randomly selected 10-seed-weight was determined. The harvested fruit numbers for these measurements were 7 and 3, for low and high temperatures, respectively.

## RESULTS

### *Pollen germination*

The pollen germination percentages at 30/25°C and 20/15°C in 1995 are shown in Table 4-2-1. High temperatures adversely affected pollen germination. Pollen development period was more heat sensitive than pollen germination. Very similar tendency was observed in 1996.

**Table 4-2-1.** Percentages of cherimoya pollen germination as affected by temperatures during pollen development or germination. Germination percentages were determined 24 hours after pollen placement on 2% agar medium containing 15% sucrose. Pollen was collected at 20/15°C on May 21 and at 30/25°C on April 6 1995, in the middle of the flowering periods.

Germination temperature	Temperature at which pollen developed	
	20/15°C	30/25°C
20°C	75.6 %	16.6 %
30°C	59.0 %	8.8 %
Significance <sup>2</sup>		
Temperature of pollen development	**	
Temperature of pollen placement	*	
Interaction	n.s.	

<sup>2</sup>: Data was analyzed by two-way analysis of variance.

n.s.: non significant; \*: P<0.05; \*\*: P<0.01.

## **Fruit set**

Pollen development temperatures greatly affected fruit set, but female organs were less affected by temperature (Table 4-2-2). High temperatures decreased fruit set percentage; the highest percentage fruit set was obtained in the "low" pollen - "low" flower pollination treatment (10 out of 10 flowers/fruit), and the lowest (3 out of 11) was in the "high" - "high" pollination treatment. The fruit set percentage with "high" developed pollen was low, as most flowers dropped in a week and fruits which continued to grow to maturity were quite irregular in shape. With the "low" developed pollen, hand-pollination to "low" flowers achieved successful fruit set and no irregular fruit, and that to "high" flowers resulted in few flower drops in a week. More seeds were produced by pollination with "low" pollen. The temperature at pollination had a lesser effect on seed number. Similar tendencies were found in 1996.

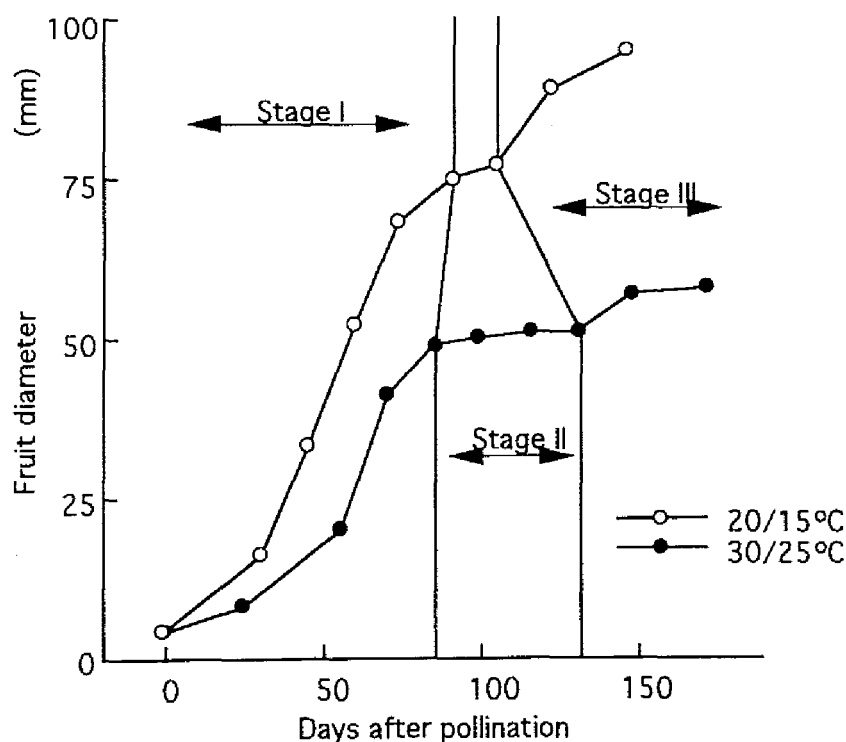
**Table 4-2-2.** Fruit set percentages and number of seeds in the mature fruit with four pollination treatments in 1995: Flowers at high day/night temperatures (30/25°C) with pollen from high temperatures (high-high), flowers at high temperatures with low-temperature (20/15°C) pollen (high-low), low-temperature flowers with high-temperature pollen (low-high) and low flowers with low pollen (low-low).

Observations		Temperature regimes of Pollen - Stigma			
		Low -Low	Low - High	High - Low	High - High
Number of flowers hand-pollinated		10	8	7	11
Number of dropped <sup>2</sup>	Flowers	0	1	4	6
	Fruitlets	0	3	1	2
	Total	0	4	5	8
Number of fruit set	Symmetrical	10	3	0	0
	Irregular	0	1	2	3
	Total	10	4	2	3
Percentage (%)	Fruit set	100	50	29	27
	Dropping in a week	0	13	57	55
	Irregular fruit	0	25	100	100
Average seed number per fruit		74	55	15	13

<sup>2</sup>: Dropped number observed before July 1 for fruitlet, within a week after pollination for flowers.

### ***Fruit growth and development***

The growth patterns of fruits at both low and high temperatures were double-sigmoid (Fig. 4-2-1 and Fig. 4-2-2). The growth curve can be divided into three phases: with a slow growth period (stage II) during which the fruit growth stopped or remained nearly constant, and two rapid growth periods (stages I and III) observed before and after stage II (Fig. 4-2-1). The stage II at high temperatures was longer, and the growth rates during stages I and III were lower. In the experiment in 1996, growth rate at stage III for fruit developed at high temperatures was accelerated when the fruit was transferred to low temperatures before stage II (Fig. 4-2-2). Fruit, which was developed at low temperatures and then transferred to high temperatures, decreased growth rate at stage III. The growth patterns of fruits kept at fixed temperature regimes throughout the season were very similar to those in 1995.



**Fig. 4-2-1.** Single fruit growth curves at 30/25 °C and 20/15 °C day/night temperature regimes. Data are typical fruit diameters at both temperatures. The diameter at pollination was 4mm. The high temperature fruit was pollinated on April 5 1995, and the cool temperature fruit on May 1. Both were harvested on September 26 1995.



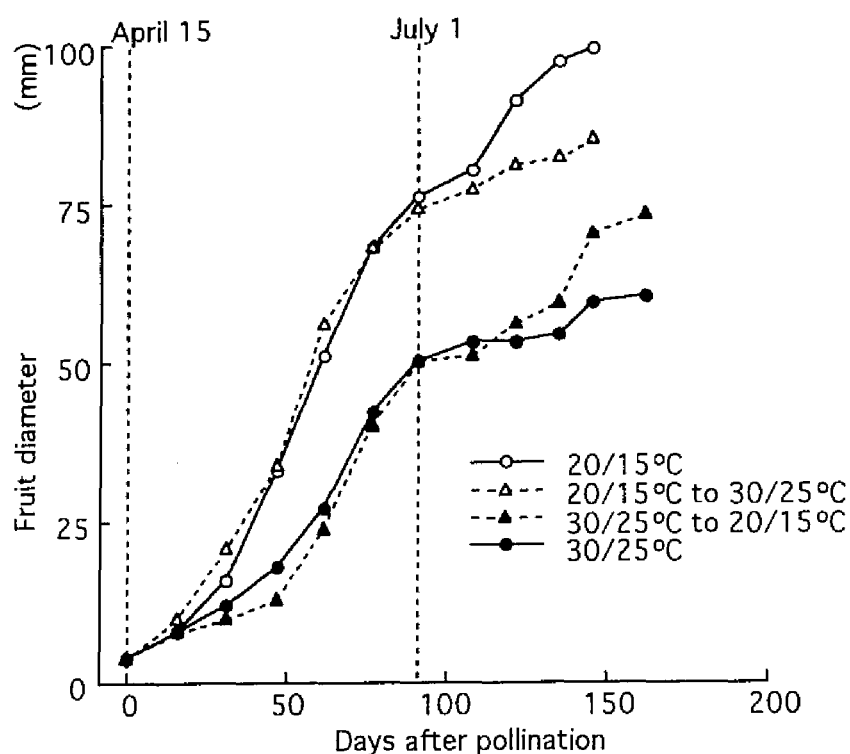


Fig. 4-2-2. Growth curve of single cherimoya fruit pollinated on April 15 1996 at 20/15°C (open) and at 30/25°C (close). Fruit were grown at the same temperatures as the fruit pollinated (circle) or transferred to another temperatures on July 1 (triangle).

Growing temperatures affected fruit size and shape, but not TSS (Table 4-2-3). Fruit was smaller and irregular in shape at high temperatures (Fig. 4-2-3). High temperatures reduced seed number, but had a marginal effect on seed weight. Fruit weight per seed was slightly decreased by high temperatures.

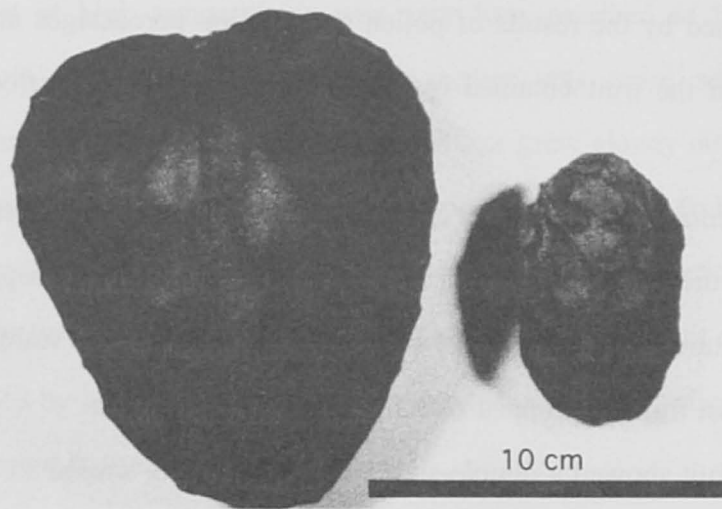
**Table 4-2-3.** Fruit quality of cherimoya as affected by 20/15°C and 30/25°C temperatures.

Observations		Temperature regimes		Significance <sup>x</sup>
		20/15°C	30/25°C	
Fresh weight of fruit (g)		468	68.1	**
Seed number per fruit		78.9	13.0	**
Weight of 10 seeds (g)		4.4	4.2	*
Fruit weight per seed (g)		5.9	5.3	n.s.
Fruit symmetry <sup>z</sup>		1.3	4.3	**
Fruit diameter (mm)	Horizontal	95.6	50.7	**
	Vertical	95.4	50.3	**
TSS <sup>y</sup> (%)		25.1	24.6	n.s.

<sup>z</sup> Fruit symmetry; Index for fruit shape; 1, highly symmetrical; 5, completely irregular.

<sup>y</sup> Percentage of total soluble solid measured by refractometer.

<sup>x</sup>: n.s., \*, \*\*: No significant, significant difference at  $p < 0.05$ ,  $p < 0.01$  by t-test, respectively.



**Fig. 4-2-3.** Cherimoya fruit harvested from 20/15°C (left) and 30/25°C (right) day/night temperature regimes. The fruit at high temperature was small and irregular in shape.

## DISCUSSION

George and Nissen (1988) found that high vapor pressure deficit reduced fruit set percentage in atemoya but temperature had no effect on fruit set. They ascribed the former to dehydration of the stigma. A similar response was noted with cherimoya; low humidity decreased pollen germination percentage and stigma receptivity (Utsunomiya *et al.*, 1992a). Accordingly, the stigma appeared to be sensitive to dehydration but to be heat tolerant. In the present experiment, stigmatic receptivity was less affected, by higher temperature, and the pollen germination percentage was decreased. Pollen is assumed to be more sensitive to heat stress than the stigma.

As cherimoya has multiple flowers, fruit symmetry is considered to be an index for estimating the degree of success in pollination. Cherimoya fruit has often been reported to be asymmetrical because the carpellary wall increases in thickness, only in those carpels which set seed (Schroeder, 1941). In this experiment, pollination with “high” pollen increased the

frequency of irregular shaped fruit (Table 4-2-2). The seed number per fruit in the “low” pollen - “high” flower pollination treatment was less than that in the “low-low” treatment. This was explained by the results of pollen germination percentages at 20°C (Table 4-2-1). The less seeds in the fruit obtained by “high” pollen might be ascribed to a lower pollen viability.

Suppressed initial fruit growth, at high temperatures, caused a reduction in harvested fruit. This was due to the growth restriction of fruit and the consequent dropping before maturity. At high temperatures, even if flowers were pollinated with high viability pollen from low temperatures, most fruitlets dropped within a month of pollination.

Cherimoya fruit showed a double-sigmoid growth curve, similar to other species such as soursop (Worrell *et al.*, 1994) and persimmon (Zheng *et al.*, 1990). However, the fruit growth suspended period (stage II) was variable, influenced by temperature. The stage II was longer at high temperatures, while it was shorter and indistinct at low temperatures. At high temperatures, longer days were required for maturity than at low temperatures. This can be attributed to a longer lag phase. A similar tendency has been noted for persimmon (Zheng *et al.*, 1990). Sugiura *et al.* (1995) found that the initial fruit growth of Japanese pear was accelerated by increasing temperature, and that the growth rate in the stage III was unaffected by temperature. In contrast, the growth rates of cherimoya fruit during both stages I and III declined at high temperatures.

In this study, high temperatures decreased fruit set and growth of cherimoya, by reducing pollen viability and then inhibiting fertilization. High temperatures also reduced fruit set and initial fruit growth. Thus, in areas where insect pollinators are absent, inevitable hand-pollination on cool cloudy or rainy days will be more advantageous. Higher temperature through the fruit growing season may adversely affect thickening growth, by extending the suspended growth.

## SUMMARY

The effects of high (30/25°C) and low (20/15°C) day/night temperatures on fruit set and

fruit growth of potted cherimoya (*Annona cherimola* Mill.) trees were investigated under sunlit glass-house conditions. Pollen germination percentage was adversely affected by high temperatures. Fruit set at high temperatures was very low, ascribed to both pollen and stigmatic damage from heat stress, although the former was more sensitive to high temperatures than female organs. Fruit at high temperatures grew slowly and required more days to mature than those at low temperatures. High temperatures reduced fruit growth rates at the initial and final growth stages. The intervening growth-deceleration period was longer at high temperatures. High temperatures produced asymmetrical and small fruit containing small number of seeds, caused by low-viability pollen. Temperature had no significant effect on the total soluble solids content in fruit.

### General discussion

Tropical and sub-tropical fruits have been enhancing growers' and consumers' interests and demand. However, the production areas of most such fruits are very limited because of narrow environmental adaptability. Some fruits which have long shelf lives are favorable to export from specified production countries in the tropics. In fact, these are good means to gain foreign currency for developing nations mostly in the tropics. On the other hand, such fruit as cherimoya is not often to be exported because of its short shelf life. However, the quality of this fruit is rated excellent and the market price is also high. Thus, the demand to introduce and cultivate cherimoya have been increasing world wide.

Fruit trees in the tropics play more important role than in temperate zones because of their multipurpose plant use. Most fruit trees in the tropics can contribute not only food supply for self-consumption but also be important and valuable cash crops even in rural areas. However, the studies on tropical fruit trees in any research areas have been behind than temperate fruit trees. It is, therefore, an urgent necessity to hasten the researches concerning environmental physiology of tropical fruit trees. Major points that environmental physiology on tropical fruit trees is contributory to tropical agriculture can be arisen as follows:

1. Sustainable agriculture in the tropics is closely related to tree crops,
2. Agriculture in the tropics has a great potential to influence global environment, and
3. The information will lead to new development of cultivation technique.

#### ***Growth response and acclimation to high temperature***

Some trial introductions of cherimoya into lowland sub-tropical or warm temperate areas have been often resulted in failure. Growth suppression by heat stress is thought to be a main

obstruction. However, few researches concerning heat stress of cherimoya have been conducted. Limited information prevents successful cultivation for this fruit tree in warm climates.

The most characteristic point of tropical crops is the growth response and the acclimation to high temperature environment. Generally, plant growth and metabolism are profoundly affected by environmental temperature (Fitter and Hay, 1981). The present experiment using *Annona* seedlings indicated lesser growth of cherimoya at 30/25°C than sugar apple. Cherimoya decreased the carbohydrate distribution to stem and root at 30/25°C. Environmental temperature around 30°C was rather high for steady plant growth for cherimoya. This fact supports cool highland tropical origin of this species. Mature cherimoya trees also exhibited gradually decreasing growth rate at 30/25°C although the initial sprouting was vigorous. This tendency is different from those previously reported for mango (Whiley *et al.*, 1989) and lychee (Menzel and Paxton, 1985). For these tree crops, the dry matter allocation to the leaves has been noted to increase at 30°C and above. Limited cherimoya growth and matter productivity was closely related to low photosynthetic activity.

In fact, cherimoya assimilation was susceptible to hot and dry conditions, because of increasing  $VPD_L$  with high temperatures and irradiance. Such environmental condition suppressed stomatal conductance (Inoue *et al.*, 1984). It is thought to be important to maintain leaf temperature at low level to decrease  $VPD_L$ . Manipulation of the aerial environment using over-tree misting or shading suggested to be effective to decrease leaf temperature during hot seasons. However, misting sometimes leads to plant disease. Thus, cherimoya growth response was compared to other commercially important *Annona* species under developmental light intensities. The results indicated that cherimoya was more shade-tolerant than sugar apple and soursop. Cherimoya  $A_C$  was slightly affected by shading levels of 25% and 45% sunlight. The vegetative growth under 45% sunlight was more vigorous than under full sunlight condition.

Plants commonly acclimate to shade through increases in  $LAR$ , which was greater in soursop and sugar apple than cherimoya. Thompson *et al.* (1992) reported that the increase in

$LAR$  was greatest for sun-loving species and least for shade-tolerant species among tropical rain forest trees. Their study supports the results in the present study that cherimoya is considered to be more shade-tolerant than other two species. Cherimoya seems to have more effective mechanism to utilize low irradiance compared with other two species. In conclusion, this study suggests that adequate levels of shading improve matter production of cherimoya during hot season.

### ***Photosynthetic adaptation to heat stress***

Leaf physiological factors affecting the reduction in the matter production under heat stress will be as follows: 1) stomatal closure, 2) leaf morphological and anatomical acclimation, and 3) direct heat inhibition to photosystem reaction center. Photo-respiration has also been reported to decrease  $A_C$  (Ishii, 1992). However, it is now not considered to be related to  $A_C$ , because the photo-respiration is the mechanism to remove active oxygen which is excessively produced in photosystem II of  $C_3$  plants under high irradiance (Kozaki and Takeba, 1996; Heber *et al.*, 1996).

Stomata impose a large limitation on  $CO_2$  assimilation rate (Farquhar and Sharkey, 1982). The stomatal closure was the main cause of the midday reduction in  $A_C$  at high temperatures in cherimoya (Fig. 3-1-4). High temperatures reduced  $g_s$  in response to high  $VPD_L$ . Stomata is related to plant water status *via* transpiration. In this result, there was little difference in the transpiration rate at midday between high and low temperatures, despite a significant difference in  $g_s$ . This was because of the off set of  $VPD_L$  effect by  $g_s$  effect. This response might be an effective mechanism to conserve water through wide range of temperatures. A decrease in  $g_s$ , however, causes excessive rise of leaf temperature at high ambient air temperatures. High leaf temperature may lead to an irreversible damage of the photosynthetic apparatus (Berry and Björkman, 1980). Such damage of photosynthetic metabolism will occur when leaf temperature exceeds  $40^\circ C$  (Kobza *et al.*, 1984). The leaf temperatures of cherimoya in this experiments (Fig. 2-1-5) and under actual orchard condition (Fig. 3-2-5) were not beyond  $40^\circ C$ . The situation of actual cultivation with such a high leaf temperature is unusual

for commercial orchard.

The degree of heat inhibition of photosystem can be detected using capacity of the ribulose biphosphate carboxylase-oxygenase (Rubisco). It is known that  $A_C$  is limited only by the capacity of the Rubisco, under a strong light intensity above  $1000 \mu \text{mol m}^{-2} \text{s}^{-1}$  and low  $C_i$  conditions (Sharkey, 1985). In this region, the slope of the  $A_C / C_i$  curve reflects the activity of the Rubisco in the leaf proportionally (Farquhar and Sharkey, 1982). In the present study, cherimoya leaves grown at high temperature were suggested to exhibit significantly low Rubisco activities. This suggests that leaves growing at 30/25°C had lowered  $\text{CO}_2$  absorption ability of those at 20/15°C, irrespective of stomatal factors. However, this reduction in the  $\text{CO}_2$  absorption ability was not necessarily brought about heat inhibition of photosystem reaction center. High temperatures resulted in a thinner leaf with less chlorophyll content. Development of palisade and mesophyll tissues was less at high temperatures. It is assumed that thinner leaves with a low chlorophyll content may represent a disadvantage for the efficient usage of light energy. Therefore, lower photosynthetic activity at high temperatures is assumed to be caused by coarse leaf structure, as well as stomatal limitation.

The reason why cherimoya leaf developed at high temperature possessed low ability of photosynthetic apparatus has not been reported. Low chlorophyll content is assumed to be a result of destruction by high temperature and high irradiance. Thin leaf is thought to be a result of acclimation to high temperature to enhance a refrigeration function of leaf. Plant leaf transpires to avoid exceeded increase of leaf temperature. Water supply depends mainly on its uptake from roots. When water uptake is below the transpiration, transpiration rate decreases, and leaf temperature increases. Such tendency is more profound with increasing temperature. Consequently, effective refrigeration mechanism is necessary with little water consumption as possible, especially under high temperature. Thin leaf at high temperature had large intercellular space in spongy layer. It is possible to consider that this morphological adaptation is advantageous to cool the leaf by sensible heat loss. This implies that cherimoya leaf with poor structure developed at high temperature is favorable to maintain leaf temperature at lower level by more vertical leaf orientation with high leaf flexibility, although such



morphological and anatomical adaptation is disadvantageous for vigorous photosynthetic response.

Shade leaves typically increase quantum use efficiency through physiological adaptations (Fitter and Hay, 1981). Under natural light intensity,  $A_C$  reduction which was similar response to that at 30/25°C temperature condition was duplicated. Easily increasing leaf temperature *via* decreasing  $g_s$  caused the decline in  $A_C$  under full sunlight condition. Stomatal closure is sometimes caused by leaf water stress (Brakke and Allen, 1995). The daytime reduction of leaf water potential was more pronounced under less shaded conditions (Fig. 3-2-9). Decreased  $g_s$  in this situation is supposed to be a water conserving mechanism at the expense of  $A_C$ . Such response in cherimoya under strong light intensity is similar under high temperature conditions. It can be concluded that shading of 64-24% sunlight was found to be effective to reduce leaf temperature and to keep high  $A_C$ , easing of water stress and preventing the danger of photoinhibition.

Now, the shading of cherimoya has been commonly introduced to many commercial orchards in southeast part of Japan.

### ***Reproductive growth response to high temperature***

Flowering, fruit-set, and fruit growth affect directly fruit production. High temperature decreased the number of flowers, diminished flower size, and shorten flowering period. Pollen viability and stigmatic receptivity were also reduced, resulting in unsuccessful fertilization. Inadequate fertilization caused asymmetrical fruit and fruit drop. High temperature also decreased fruit growth. Fruit at high temperature required long period to mature, although internal quality was not affected by temperature. These responses were considered to be resulted from reduction in matter production at high temperatures and direct heat inhibition to reproductive organs.

It has been reported that high temperatures over 30°C reduced or inhibited the floral induction of tropical and sub-tropical fruit trees such as litchi, mango, avocado, and pummelo. In cherimoya, although flowers were largely reduced at 30/25°C, the number of basal flowers

was less affected by such temperature regimes. Flowers at high temperatures became significantly smaller and the developmental period was shorter than at low temperatures. This was resulted from rapid growth of floral organs by high temperatures. Thus, high temperatures resulted in an early and short flowering season.

Floral morphogenesis occurred both in the long-dormant axillary buds and in the terminal buds of the extending shoots at low temperatures, whereas that occurred only in the axillary buds at high temperatures. This indicates that optimal temperature for floral differentiation around the terminal buds is lower than 30/25°C. On the other hand, basal flowers which were produced in the axillary buds were induced even at 30/25°C, although the number was small. Floral induction through two years may lead to an expectation of every year flowering even at 30/25°C.

The better fruit production requires the success in fertilization. Pollen viability and stigma receptivity are the main two factors related to fertilization. The pollen germination percentage decreased at high temperatures. Pollen was more sensitive to heat stress than stigmas. A large quantity of pollen of high viability are considered to be indispensable to a promising hand-pollination. To attain sufficient viable pollen, it is recommended to cultivate cherimoya in relatively cool weather conditions through the flowering season. In areas where insect pollinators are absent, hand-pollination on a cloudy or rainy day will be inevitable. Moreover, pollen collected from flowers which were harvested in early morning and come to the anthesis at room temperature around 20°C will be effective to improve fruit-set.

High temperatures decrease fruit-set and fruit growth of cherimoya. Higher temperature throughout the fruit growing season may adversely affect the growth and development. Reduction in the number of fruit at harvest at high temperatures probably resulted from depressed initial fruit growth during fruitlet periods, as well as the low rate of success in fertilization. Even though the fruit contained a large number of seeds by a successful pollination using vigorous pollen, its growth was restrained under high temperature conditions, and consequently a number of fruit dropped before maturity. This suggests that high temperatures limited directly fruit growth and development. Thus, covering individual fruit to

shade or bearing fruit inside canopy will be recommended to alleviate heat suppression of fruit growth.

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